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2018 IIBC
UNIVERSITAT
JAUME I

Castellón (Spain) 20 - 22 June 2018



6th International Iberian Biophysics
Congress

X Iberoamerican Congress of
Biophysics

*book
of* **Abstracts**

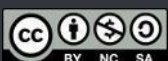
EDITORS:

Jesús Salgado

Jorge Alegre-Cebollada

Xavier Daura

Teresa Giráldez



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2018 IIBC

UNIVERSITAT
JAUME I

Invitation Letter

Dear Colleagues,

On behalf of the Organizing Committee, it is my pleasure to invite you to attend the 6th International Iberian Biophysics Congress and X Iberoamerican Congress of Biophysics. This international conference has a tradition of almost two decades. The 2018 edition is organized under the auspices of the Spanish Biophysical Society (SBE), the Portuguese Biophysical Society (SPBf) and the Latin American Federation of Biophysical Societies (LAFeBS). IIBC–2018 will be held in Castellón (Spain) on 20–22 June 2018, in the campus facilities of Universitat Jaume I.



The scientific program includes several Plenary Lectures, as well as Parallel Symposia on selected topics covering the main research areas of Biophysics. Symposia will host invited talks and also short communications selected from submitted abstracts with preference for young researchers. Following the tradition of previous Meetings, a New and Notable Workshop will take place in the morning of the first day. The organizers are committed to make the Poster Sessions a place for networking and the occasion of fruitful and lively discussions in a relaxed atmosphere. Reduced registration fees will apply to participants who are SBE members. Moreover, a number of grants sponsored by SBE and SPBf will be available to encourage young researchers' participation.

Please, see also the satellite summer school MemBiophysics 2018, to be held from 25 to 29 June 2018 in FFUP/ICBAS complex, Oporto, Portugal.

Best regards,

VICENTE M. AGUILERA

Chair of the Organizing Committee



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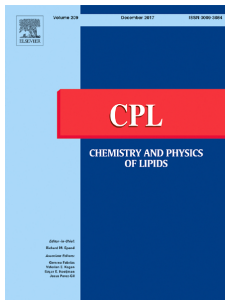
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NN | New and Notable Biophysics

Hall Auditorium

Chair 1 MANUELA PEREIRA

Chair 2 MARÍA F. GARCÍA-PARAJÓ

Date: Wednesday, June 20th

Time: 11:00–13:10

NN.1 Structural basis for energy transduction by respiratory alternative complex III

MANUELA PEREIRA. §

Instituto de Tecnologia Química e Biológica – António Xavier, Universidade Nova de Lisboa, ITQB NOVA, Oeiras, Portugal and Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

§ mpereira@itqb.unl.pt

Acquisition of energy is central to life as all organisms depend on constant energy transduction mechanisms to grow and reproduce themselves. In this regard Prokaryotes are amazingly versatile and robust organisms. They have an adaptable metabolism and present a diversity of respiratory chains, allowing the use of a plethora of different electron donors and acceptors. We explored the diversity of membrane respiratory chains and the presence of the different enzyme complexes in the several phyla of life. We performed taxonomic profiles of the several membrane bound respiratory proteins evaluating the presence of their respective coding genes in all species deposited in KEGG database. We analyzed 26 quinone reductases, 5 quinol:electron carriers oxidoreductases and 18 terminal electron acceptor reductases. We concluded Chemiosmosis is present in all organisms. We have been also investigating respiratory complexes at the protein level, as exemplified by our studies on Alternative Complex III (ACIII), a new quinol:cytochrome *c*/HiPIP oxidoreductase. ACIII is a multisubunit complex with four transmembrane subunits and three peripheral subunits a pentaheme and a monoheme cytochromes and a large [Fe-S] clusters containing subunit. We have been performing a thorough functional and structural characterization of ACIII, using several complementary biochemical and biophysical approaches. We have recently obtained a 3.9 Å structure of ACIII by single-particle cryo-electron microscopy, which provides novel insights into a mechanism for energy transduction and introduces ACIII as a redox-driven proton pump.

NN.2 Acyl chain asymmetry and polyunsaturation of brain phospholipids facilitate membrane vesiculation without leakage.

MARCO M. MANNI,^{1,2,§} M. L. Tiberti,² S. Pagnotta,³ H. Barelli,² R. Gautier,² B. Antony.²

¹Instituto Biofisika (UPV/EHU, CSIC), Leioa (SPAIN); ²Institut de Pharmacologie Moléculaire et Cellulaire (CNRS, UNS), Valbonne, France; ³l'Université Nice Sophia Antipolis (UNS).

§marco.manni@ehu.eus

Phospholipid membranes form cellular barriers but need to be flexible enough to divide by fission. Phospholipids generally contain a saturated fatty acid (FA) at position *sn1* whereas the *sn2*-FA is saturated, monounsaturated or polyunsaturated. Our understanding of the impact of phospholipid unsaturation on membrane flexibility and fission is fragmentary. Here, we provide a comprehensive view of the effects of the FA profile of phospholipids on membrane vesiculation by dynamin and endophilin. Coupled to simulations, this analysis indicates that: (i) phospholipids with two polyunsaturated FAs make membranes prone to vesiculation but highly permeable; (ii) asymmetric *sn1*-saturated-*sn2*-polyunsaturated phospholipids provide a tradeoff between efficient membrane vesiculation and low membrane permeability; (iii) When incorporated into phospholipids, docosahexaenoic acid (DHA; omega-3) makes membranes more deformable than arachidonic acid (omega-6). These results suggest an explanation for the abundance of *sn1*-saturated-*sn2*-DHA phospholipids in synaptic membranes and for the importance of the omega-6/omega-3 ratio on neuronal functions.

NN.3 Advances and pitfalls in computational enzymatic catalysis

MARIA JOÃO RAMOS.[§]

UCIBIO@REQUIMTE, Department of Chemistry and Biochemistry Faculty of Sciences, University of Porto, Porto, Portugal.

§mjramos@fc.up.pt

The establishment of enzyme reaction mechanisms in systems with S-S bonds, using QM/MM techniques [1,2,3] will be addressed, emphasizing the methodological aspects that have to be taken into consideration to achieve accurate and reliable results. The role of enzyme flexibility on catalytic rates will be discussed too.

References

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3. Sousa, Ribeiro, Neves, Brás, Cerqueira, Fernandes, Ramos, *WIREs Computational Molecular Science* (2016) 7: 1281.

NN.4 Separating actin-dependent chemokine receptor nanoclustering from dimerization indicates a key role for clustering in CXCR4 signaling and function

LAURA MARTÍNEZ-MUÑOZ,^{1,2,§} José Miguel Rodríguez-Frade,¹ Rubén Barroso,¹ Carlos Óscar S. Sorzano,³ Juan A. Torreño-Pina,⁴ César A. Santiago,⁵ Carlo Manzo,^{4,6} Pilar Lucas,¹ Eva M^a García-Cuesta,¹ Enric Gutierrez.⁴

¹Chemokine Signaling Group, Department of Immunology and Oncology, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain; ²Department of Cell Signaling, Centro Andaluz de Biología Molecular y Medicina Regenerativa, Spain; ³Biocomputing Unit, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain; ⁴ICFO-Institut de Ciències Fòniques, The Barcelona Institute of Science and Technology, Barcelona, Spain; ⁵X-ray Crystallography Unit, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain; ⁶Universitat de Vic, Universitat Central de Catalunya (UVic-UCC), Vic, Spain.

§laura.martinez@cabimer.es

A current challenge in cell motility studies is to understand the molecular and physical mechanisms that govern chemokine receptor nanoscale organization at the cell membrane, and their influence on cell response. Using single-particle tracking and super-resolution microscopy, we found that the chemokine receptor CXCR4 forms basal nanoclusters in resting T cells, whose extent, dynamics and signaling strength are modulated by the orchestrated action of the actin cytoskeleton, the co-receptor CD4 and its ligand CXCL12. We identified three CXCR4 structural residues that are crucial for nanoclustering, and generated an oligomerization-defective mutant that dimerized but did not form nanoclusters in response to CXCL12, which severely impaired signaling. Overall, our data provide new insights to the field of chemokine biology by showing that receptor dimerization in the absence of nanoclustering is unable to fully support CXCL12-mediated responses, including signaling and cell function *in vivo*.



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Opening Lecture

Hall Auditorium

Chair VICENTE M. AGUILLELLA

Date: Wednesday, June 20th

Time: 16:00–16:45

PL.1 Single-molecule biophysics: new insights into the dynamics of escape from potential and entropic traps

SERGEY M. BEZRUKOV.[§]

NICHD, National Institutes of Health, MD, USA.

[§]bezrukos@mail.nih.gov

Dr. Sergey Michael Bezrukov received his M.S. in physics from St. Petersburg Polytechnic University, Russia, his Ph.D. in biophysics from Moscow State University, and a D.Sci. in physics and mathematics from the Russian Academy of Sciences. He started his career as a researcher at the St. Petersburg Nuclear Physics Institute in Russia and moved to United States in 1990. In 1992, he joined the National Institutes of Health (NIH) as a visiting scientist and was awarded tenure by NIH in 2002. Dr. Bezrukov's honors include election to Fellowship in the American Physical Society (2009) and the NIH Director's Award in Science and Medicine (2010). Since October 2002 he is the Chief of the NICHD/NIH Section on Molecular Transport, which combines physical theory with experiments on bacterial, mitochondrial, and toxin-induced membrane channels, reconstituted in planar lipid bilayers. This line of research serves as the basis for the development of new approaches to treatment of diseases where regulation of transport through ion channels plays the key role.



Problems of barrier crossing dynamics in systems ranging from relatively simple isomerization reactions to channel-facilitated transport to protein folding are among the hot topics of single-molecule biophysics. These dynamics are frequently modeled as one-dimensional diffusion of a particle in a potential of mean force. To gain deeper insights into escape processes, we analyze the “fine structure” of particle trajectories in such systems. Specifically, we divide the trajectories into two segments: a looping segment, when the particle unsuccessfully tries to escape returning to the trap bottom, and a direct-transit segment, when it finally escapes moving without returning to the bottom. By using both an analytical approach and Brownian dynamics simulations, we show that the force/potential dependences of the two corresponding mean times are qualitatively different. The mean looping time monotonically increases with the external force pushing the particle to the trap bottom or with the increasing entropic barrier at the trap exit. In contrast to this intuitively appealing result, the mean direct-transit time shows rather counterintuitive behavior: it is reduced by force application independently of whether the force pushes the particles to the trap bottom or to the trap exit. These findings allow for a fresh look at the experimental observations.

RSEF-SBE Lecture

Hall Auditorium

Chair ANTONIO FERRER-MONTIEL

Date: Thursday, June 21st

Time: 12:00–12:45

PL.2 Electrical signaling in bacteria

JORDI GARCÍA-OJALVO.[§]

Universitat Pompeu Fabra, Barcelona, Spain.

[§]jordi.g.ojalvo@upf.edu

Dr. Jordi Garcia-Ojalvo obtained his PhD in statistical physics at the University of Barcelona in 1995. He did postdoctoral work at the Georgia Institute of Technology in Atlanta in 1996, working on laser dynamics, and at the Humboldt University of Berlin in 1998 as an Alexander von Humboldt Fellow, studying noise effects in excitable media. He was IGERT Visiting Professor at Cornell University in Ithaca, New York, in 2003, at which time he began working in the field of systems biology. In 2008 he became Full Professor at the Universitat Politècnica de Catalunya, where he had been teaching applied physics since 1991. He is Visiting Research Associate in Biology at the California Institute of Technology since 2006, and joined the Universitat Pompeu Fabra in October 2012.



Most of what is known of the structure of eukaryotic ion channels has resulted from studies of bacterial ion channels. Yet the function of these membrane proteins in their original prokaryotic hosts has remained mostly unclear so far. In this talk I will present an overview of our recent work in bacterial biofilms, which has revealed that these organisms use ion channels to communicate their stress levels via electrical signaling. This strategy enables biofilms to self-organize in space and time, ensuring their viability in the presence of limited nutrients and their resistance to external attacks. We will discuss how these dynamical strategies are generated, and how they can exist even under cell-cell heterogeneity.

LAFeBS Lecture

Hall Auditorium

Chair JUAN CARMELO GÓMEZ-FERNÁNDEZ

Date: Thursday, June 21st

Time: 18:15–19:00

PL.3 Photo-oxidation in membrane and cell biophysics: combining fluorescence, X-ray scattering () and microscopy techniques

ROSANGELA ITRI.[§]

IFUSP, Universidad de São Paulo, São Paulo, Brazil.

[§]itri@if.usp.br

Dr. Rosangela Itri holds a degree in Physics from the Pontifical Catholic University of São Paulo (1982), a Masters in Physics from the University of São Paulo (1986) and a PhD in Physics from the University of São Paulo (1991). She is currently prof. Titular of the University of São Paulo. She has experience in the area of Physics, with emphasis on Structure of Liquids and Solids; Crystallography and Biophysics mainly focusing on the following topics: SAXS, model membranes (LUVs and GUVs), photodynamic therapy, proteins in solution, amyloid proteins, membrane and protein photooxidation, magnetic nanoparticles as carriers of biologically active molecules and interaction of toxins, peptides and alkylphospholipids with lipid membranes. She is a full member of the International Scientific Committee of LNLS and Scientific Director of the Brazilian Society of Biophysics.



Lipid oxidation can disturb the structure and function of cells, which may have pathologic consequences as Parkinson's and Alzheimer diseases, diabetes, among others. We will firstly show how lipid chemical transformations induced by oxidative stress alter plasma membrane structural features. SAXS results from lipid vesicles composed of different amounts of unsaturated, oxidized and saturated lipids will be presented. The SAXS data analysis points out to an increase in membrane surface area of hydroperoxidized lipid bilayers, in good agreement with micropipette measurements on giant unilamellar vesicles (GUVs) under photo-oxidation observed by optical microscopy (MO) and molecular dynamic simulation results. Further, SAXS also allows us localizing the oxidized species inside the membrane. In addition, changes in biophysical parameters as elastic modulus and membrane packing promoted by lipid oxidation as well as raft organization are also explored by MO and fluorescence techniques. We then extend our study to mimetic membranes of lysosomes with the aim to explore how lipid oxidation may impact on cell death associated to autophagy. Finally, changes in the mechanical properties of oxidized Red Blood cell membrane are evaluated by newly designed micropipette manipulation technique for dynamic membrane elasticity measurement.

Plenary Lecture

Hall Auditorium

Chair JOSÉ MIGUEL MANCHEÑO

Date: Friday, June 22nd

Time: 11:30-12:00

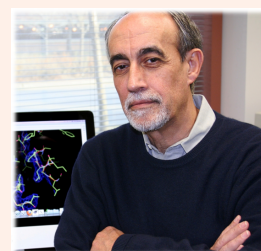
PL.4 From altar boy to sorcerer's apprentice: biophysics and beyond

CELERINO ABAD-ZAPATERO.[§]

University of Illinois at Chicago (UIC), IL, USA.

[§] caz@uic.edu

Dr. Celerino Abad-Zapatero obtained a degree in Physics from the University of Valladolid, Spain, and later a Ph.D. in Biophysics (Macromolecular Crystallography) from the University of Texas at Austin, sponsored by a Fulbright Scholarship. He was part of the team that unveiled the atomic structures of the first viruses (SBMV) in the early 1980's with Michael Rossmann at Purdue University. He worked in the pharmaceutical industry (Abbott Laboratories) for twenty-two years using the methods of Structure- Based Drug Design to expedite the design and optimization of medicinal drugs. He retired from the private industry in 2008 and is currently Adjunct Professor to the Graduate Faculty at the University of Illinois at the Chicago Campus.



Many of the exciting scientific discoveries related to the origin of modern Molecular Biophysics and in particular, macromolecular crystallography, happened 'very far' away from Spain. The iconic structure of DNA was unveiled to the world in 1953, fourteen years after the end of the Spanish Civil war (1936-1939), when the Spanish scientific and academic communities were completely isolated from the rest of the European nations. Only in the late 1950's and 1960's, when the children born after the Spanish civil war reached adulthood, there appeared to be a window of opportunity for those individuals to expand their scientific horizons. The presentation will reflect on the thoughts, experiences and modest contributions of one of those individuals, intertwined with the extraordinary achievements and developments in macromolecular crystallography. This is the area Molecular Biophysics that has provided the scientific and biomedical community with an unimaginable richness of atomic detail about the molecules that make life possible. The notion will be presented that there are novel areas of multidisciplinary 'biophysical' research related to medicinal drug discovery, and connected to the vast amounts of existing atomic macromolecular structures accumulated by the efforts of more 'classical' biophysicists.

A file with this presentation is available from: [https://caz.lab.uic.edu/discovery/Cele_Abad-Zapatero - IIBC2018-final.pdf](https://caz.lab.uic.edu/discovery/Cele_Abad-Zapatero_IIBC2018-final.pdf). To be cited as "From Altar Boy to Sorcerer's Apprentice: Biophysics and Beyond". Lecture by C. Abad-Zapatero at the 6IIBC-2018 Congress.

Closing Lecture

Hall Auditorium

Chair JESÚS PÉREZ-GIL

Date: Friday, June 22nd

Time: 18:00-18:45

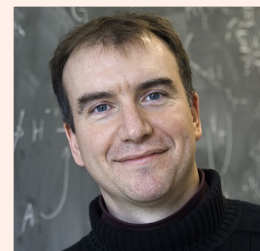
PL.5 Phase transitions and the principle of detailed balance in living systems

FREDERICK C. MACKINTOSH.[§]

Rice University, Houston, TX, USA.

[§]fcmack@gmail.com

Dr. Fred C. MacKintosh received his Ph.D. in Theoretical Physics from Princeton University in 1989. Following a postdoctoral fellowship at Exxon Corporate Research, he began his academic career in the University of Michigan's Physics Department, first as an Assistant and then Associate Professor. In 2001, he joined the Physics faculty of Vrije Universiteit in Amsterdam, as Professor of Theoretical Physics. His key achievements include: the development of commonly used models of elasticity and dynamics of biopolymer gels, combined experimental and theoretical advances in micro rheology and non-equilibrium, motor-activated gels and active diffusion in cells, as well as the identification of affine to non-affine transitions and critical behavior in fiber networks.



The mechanics of cells and tissues are largely governed by scaffolds of filamentous proteins that make up the cytoskeleton, as well as extracellular matrices. Evidence is emerging that such networks can exhibit rich mechanical phase behavior. A classic example of a mechanical phase transition was identified by Maxwell for macroscopic engineering structures: networks of struts or springs exhibit a continuous, second-order phase transition at the *isostatic* point, where the number of constraints imposed by connectivity just equals the number of mechanical degrees of freedom. We will present recent theoretical predictions and experimental evidence for mechanical phase transitions in both synthetic and biopolymer networks. Living systems typically operate far from thermodynamic equilibrium, which affects both their dynamics and mechanical response. As a result of enzymatic activity at the molecular scale, living systems characteristically violate detailed balance, a fundamental principle of equilibrium statistical mechanics. We discuss violations of detailed balance at the meso-scale of whole cells.

IV

Symposia

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S0 | Physics in Biology

Hall Auditorium

Chair 1 ANTONIO ALCARAZ

Chair 2 ARMINDO SALVADOR

Date: Wednesday, June 20nd

Time: 17:15–19:15

S0.1 Physical virology: how physics can help us understand viruses

DAVID REGUERA.[§]

Universitat de Barcelona, Barcelona, Spain.

[§]dreguera@ub.edu

Viruses are fascinating biological entities, in the fuzzy frontier between life and inert matter. Despite the lack of sophisticated biological machinery viruses have found the way to efficiently infect the host, replicate, and egress the cell following, in many cases, a coordinated sequence of passive and spontaneous processes. This is a strong evidence that, during their life cycle, viruses must rely on general physical mechanisms to succeed in their different tasks and to achieve the required resistance against possible extreme environmental conditions. The search for those mechanisms and their thriving potential applications has stirred the appearance of the emerging field of Physical Virology. In this talk, I will summarize how ideas and techniques from physics can help us understand how viruses work. In particular, I will discuss the remarkable physical principles behind the architecture, self-assembly, and mechanical properties of viruses. The understanding of the biophysical mechanisms that are common to a wide class of viruses could lead to the development of novel broad-spectrum routes to attack viral infections. In addition, this knowledge is opening the door to innovative biomedical and nanotechnological applications of viruses.

S0.2 Time-lapse bioimage analysis solved as a cellular morphodynamics model fitting problem

JORGE CARNEIRO,[§] Pedro Silva.

Instituto Gulbenkian de Ciência, Oeiras, Portugal.

[§]jcarneir@igc.gulbenkian.pt

Data analysis is the major challenge and rate limiting step of bioimaging by advanced microscopy techniques. We recast the traditional problem of image analysis involving a series of painstaking and subjective procedures of image processing, noise filtering, deconvolution, segmentation, ROI definition and selection, and measurement and tracking of cells as a problem of non-linear model fitting to imaging data. It will be shown that the latter problem can be successfully solved by defining a morphodynamical model that captures the relevant physics and biology of a cell, and by estimating the parameters of this model from raw, unprocessed imaging data.

S0.3 Experimental measurement of information-content in nonequilibrium systems

FÈLIX RITORT,[§]

Facultat de Física, Departament de Física de la Matèria Condensada, Universitat de Barcelona, Barcelona, Spain and Ciber-BBN of Biomaterials and Nanomedicine, ISCIII, Madrid, Spain.

[§]fritort@gmail.com

Biology is intrinsically noisy at all levels, from molecules to cells, tissues, organs, communities and ecosystems. While thermodynamic processes in ordinary matter are driven by free-energy minimization, living matter and biology delineate a fascinating nonequilibrium state predominantly governed by information flows through all organizational levels. Whereas we know how to measure energy and entropy in physical systems we have poor knowledge about measuring information-content in general. Recent developments in the fields of stochastic thermodynamics and thermodynamic-information feedback combined with single molecule experiments show the way to define information-content in nonequilibrium systems. In this talk I will describe how to measure information-content in two classes of nonequilibrium systems. First, I will introduce the Continuous Maxwell Demon, a new paradigm of information-to-energy conversion, and demonstrate how work extraction beats the Landauer limit without violating the second law. Next, I will demonstrate the validity of a fluctuation theorem in nonequilibrium systems under continuous-time feedback and show how to measure information-content in such conditions. Second, I will introduce a mutational ensemble of DNA hairpin folders and show how to measure information-content in this context. A definition of information-content applicable to generic disordered populations is proposed. All results are experimentally verified in single molecule pulling experiments.

S0.4 Electrostatic charging of lipid membranes by ATP

HORIA PETRACHE,[§] Ryan Lybarger, Bruce Ray.

Indiana University Purdue University Indianapolis, Indianapolis, IN, USA.

[§]hpetrach@iupui.edu

Phospholipids (lipids in short) found in biological membranes are also of significant interest in material research due to their distinctive properties. Due to van der Waals attraction, lipid membranes tend to form multilamellar vesicles (MLVs) with repeat spacings (D-spacings) on the order of ten to a few hundred nanometers. D-spacing values depend not only on lipid type but also on the composition of water or buffer solution in which membranes are formed. Adenosine triphosphate (ATP), the molecule involved in the energy transfer in biological processes offers an interesting case study for tuning lipid membrane interactions. Using small-angle x-ray scattering (SAXS), NMR spectroscopy, and dynamic light scattering (DLS), we investigate lipid interactions in the presence of ATP and its hydrolysis products. We find that ATP binds to phospholipid surfaces as seen by standard NMR methods as well as by a net charging effect of membranes measured by SAXS and DLS. Interestingly, membrane charging caused by ATP gives rise to an unbinding transition of lipid multilayers which is not seen in the presence of monovalent salts. These findings can help quantitate energy transfers in biomembrane processes with possible applications to bioinspired materials.

S0.5 A comparative study between the limiting diffusion coefficients of α -amino acids and α,ω -amino acids in water and sodium chloride aqueous solutions at 298.15 K

CARMEN M. ROMERO,^{1,§} Ginna P. Trujillo,¹ Diana M. Rodríguez,¹ Ana C. F. Ribeiro,² Miguel A. Estesó.³

¹*Universidad Nacional de Colombia, Bogotá, Colombia;* ²*University of Coimbra, Coimbra, Portugal;* ³*Universidad de Alcalá.*

[§]cmromeroi@unal.edu.co

Research on interactions involving α,ω -amino acids in aqueous solutions is not extensive. In particular, the information about the diffusion of amino acids in water is very scarce and no information has been found in the literature either for the diffusion coefficient of 5-aminopentanoic acid and 6-aminohexanoic acid in water or about the effect of electrolytes on the diffusion of α,ω -amino acids in aqueous solution. For this reason, we have started a comprehensive study of the diffusion of these compounds in aqueous solutions at 298.15K using the Taylor dispersion technique. Moreover, the relative position occupied by both groups (carboxyl and amino) is being reflected in important changes in the diffusion process of these species and in differences in the value of their diffusion parameters. Hence, in the present communication we present experimental values of the limiting diffusion coefficients, D_0 , and the hydrodynamic radii, R_h (estimated from the Stokes Einstein equation), of both α -amino acids and the corresponding α,ω -amino acids in water and 0.15 mol kg⁻¹ NaCl aqueous solution, at 298.15 K. The comparison between these two data series is discussed in terms of the position of the ionic groups in the hydrocarbon chain and the NaCl effect.

S0.6 Nanostructured films based on CTPR proteins

DANIEL SÁNCHEZ-DE-ALCÁZAR-MELENDO,^{1,§} José Castro,² Juan Cabanillas-González,² Verónica Fernández-Luna,³ Rubén D. Costa,³ Susana Velasco-Lozano,⁴ Fernando López-Gallego,⁴ Aitziber L. Cortajarena.¹

¹*CICbiomaGUNE, San Sebastián, Spain;* ²*IMDEA nanociencia, Madrid, Spain;* ³*IMDEA Materiales, Madrid, Spain;* ⁴*Instituto de Síntesis Química y Catálisis Homogénea, Universidad Zaragoza, Zaragoza, Spain.*

§dsanchezdealcazar@cicbiomagune.es

Nowadays state-of-the-art in biotechnology is capable of manipulating the physico-chemical properties at molecular scale. Bottom-up design of complex functional nanostructures is of the great interest because it allows to synthesize organized structures using the intrinsic self-assembly properties of simple components. In this sense, repeat proteins are useful tools for this task due to their modular and hierarchical structure that can be the basis to construct complex supramolecular assembly. Previously, we have reported the formation of self-assembled protein films with proteins organized due to head-to-tail and side-to-side interaction, providing protein directionality. Here, we take a step beyond generating functional films based on repeated proteins with applicability in different fields such as optics, heterogeneous catalysis, or electronics. In particular, we show the formation of patterned protein films labeled with fluorescent dyes to produce Amplified Spontaneous Emission (ASE) relevant for sensing. In addition, we organize fluorescent proteins within the film to enhance the efficiency and stability of bio-light-emitting diodes (bioLEDs) extending the lifetime of the device. Finally, the films are used to entrap the enzyme catalase, without apparent loss of activity. This film is used to generate energy through the enzymatic activity by coupling the film to a piezoelectric device sensitive to the pressure changes of the produced oxygen.

S0.7 Effect of infrared light on protein conformation, adhesion and enzymatic performance.

MAGDALENA KOWACZ,[§] Piotr Warszyński.

Jerzy Haber Institute of Catalysis and Surface Chemistry, Polish Academy of Sciences, Krakow, Poland.

§dudek06@wp.pl

Adsorption of proteins at a solid surface affects characteristics of the surface (e.g. its biocompatibility) and functionality of the immobilized biomacromolecules. The latter is defined by the type of binding sites, protein conformation and its structural flexibility that enable functional motions to occur. Protein motions are only possible at certain level of hydration. We present the potential of a remote physical trigger – a non-ionizing infrared radiation (IR) to affect protein-surface interactions and conformation of adsorbed proteins. The results of FTIR spectroscopy studies indicate that IR can protect proteins from denaturation on adsorption to the solid surface. Specifically it prevents 3_{10} helical motif from unfolding. This protective effect depends on the presence of strongly hydrated amino acid residues, that in the studied cases provide also binding sites of a protein to the surface. Hydration-related supporting of the native fold further aids protein functionality. We show, using UV-vis assay, that IR indeed promotes esterase-like activity of serum albumin. IR facilitates also displacement of surface active species adsorbed to protein apolar compartments that could otherwise promote denaturation. Apart from supporting native conformation, their removal increases protein-water interfacial tension and promotes protein adhesion to the solid surface (as evidenced by results from Quartz Crystal Microbalance).

S0.8 Single-molecule characterization of heterogeneous DNA ensembles

ALVARO MARTÍNEZ-MONGE,^{1,§} Danny Incarnato,² Anna Alemany,³ Marco Ribezzi-Crivellari,⁴ Fèlix Ritort.¹

¹*Departament de Física de la Materia Condensada, Universitat de Barcelona, Barcelona, Spain;* ²*Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy;* ³*Hubrecht Institute for Developmental Biology and Stem Cell Research, Utrecht, The Netherlands;* ⁴*ESPCI Paris Tech, Paris, France.*

§alvaromartinez@ub.edu

Heterogeneity exists across all spatial scales, from communities down to molecular level. The characterization and quantification of heterogeneous effects, although have been usually overlooked, have turned out to be fundamental in many scientific disciplines, such as cancer research. By combining single-molecule measurements using optical tweezers with fluctuation theorems we have built a theoretical background that allows us to characterize a heterogeneous ensemble of DNA hairpins. We show how by averaging the work probability distributions obtained in an experimental set of bidirectional pulling experiments on a statistical sample of the heterogeneous ensemble (i.e. a simple random sampling of the whole molecular ensemble), leads to a breakdown of the fluctuation theorem symmetry through an effective temperature higher than the bath temperature. The quantification of the effective temperature allows us to define a thermodynamic quantity that contains information about the folding free energy of the ensemble. The effective temperature is physically interpreted in terms of an information content (that can be inferred rather than measured) due to the non-equilibrium sampling of the DNA sequences that form the molecular ensemble.

S0.9 Titin hydrogels to examine emergent mechanical properties of striated muscle

CARLA HUERTA-LÓPEZ,[§] Diana Velázquez-Carreras, Andrea Rodríguez-Blanco, Ricardo Esteban-González, Elías Herrero-Galán, Jorge Alegre-Cebollada.

Spanish National Centre for Cardiovascular Research (CNIC), Madrid, Spain.

§chuerta@cnic.es

Proper function of striated muscle depends on its mechanical properties. Single-molecule approaches showed the passive elasticity of muscle is determined by the mechanics of proteins, such as titin. However, due to the complexity of muscle, there are no models that can predict macroscopic mechanical behaviour from the properties of constituent proteins. Therefore, it remains a challenge to synthesize materials with the properties of muscles. We propose a bottom-up approach to produce titin-based hydrogels that recapitulate the passive elastic properties of muscle. Our approach shows a more direct correlation between the mechanical properties of the hydrogels and their constituent proteins, measured at the single-molecule level by AFM. Hydrogels are engineered via photocatalytic crosslinking of tyrosine residues present in titin immunoglobulin-like domains. Hydrogel stiffness is then measured using a custom-built stretching device. Through biochemical modification of the constituent proteins, we determine how changes in the nanomechanical properties of the protein building blocks translate into macroscopic changes in the mechanics of the resulting hydrogel. Thanks to our new strategy, it will be possible to translate the mechanical properties of proteins to higher order arrangements. Furthermore, these hydrogels represent an initial step in the establishment of biomaterials that mimic the adjustable mechanics of muscles.

S1 | Protein Structure, Dynamics and Function

Hall Auditorium

Chair 1 IRENE DÍAZ-MORENO

Chair 2 PAULA GAMEIRO

Date: Thursday, June 21st

Time: 09:00–11:50

S1.1 Targeting the solvent accessible surface area (*sasa*) in proteins

JOSE MARÍA DELFINO,[§] Evangelina Marisel-Bernar, Gabriela Elena Gómez.

Departamento de Química Biológica e IQUIFIB (UBA-CONICET), Universidad de Buenos Aires, Buenos Aires, Argentina.

[§]delfino@qb.ffyb.uba.ar

The hydrophobic effect is widely regarded as the major driving force behind folding and interactions in proteins. Consequently, SASA and its counterpart, the excluded volume, become topographic descriptors of a given folding state. However, these fundamental signatures elude straightforward experimental scrutiny. With the aim at evaluating the size, location and nature of SASA, we introduced a photochemical approach mimicking the role played by the water solvent. The rather indiscriminate reaction of diazirine (DZN), a minute CNN heterocycle, with the polypeptide chain leaves a methylated trace as the new observable. Quantitative assessment of the extent of modification is possible through metrics based on radiochemical or mass spectrometric techniques. Unlike the former, the latest multidimensional NMR advance does not demand cleavage into peptides or amino acids to pinpoint the locale of labeling. A combined analysis, including ¹H-NMR, ¹H-¹⁵N-HSQC and ¹H-¹³C-HSQC spectra, was carried out on the paradigm of *E. coli* thioredoxin (TRX). Methylation of amino acid side-chains represents the prevailing phenomenon, bearing dissimilar influence on backbone amide environments. Furthermore, inner surfaces such as those delimiting the boundaries of cavities, become prime targets for DZN. The fully consistent picture emerging from this footprint on solvent exposure illuminates relevant traits of protein conformation.

S1.2 Study of T7 core proteins involved in DNA ejection: GP15 and GP16

MAR PÉREZ-RUIZ,[§] Mar Pulido-Cid, Ana Cuervo, José L. Carrascosa.

CNB-CSIC, Madrid, Spain.

[§]mmperez@cnb.csic.es

The understanding of the mechanism of T7 genome translocation inside to the Gram negative host involves the study of two viral complexes: the tail and the core. T7 belongs to the *Podoviridae* family and it has a short and non-contractile tail that is not able to puncture the bacterial wall. It has been postulated that during infection, the tail interacts with an internal head complex, the core, to form a channel through the bacterial membrane (Hu *et al.* 2013). In this work we show the results from the purification and characterization of two main core proteins: gp15 and gp16. Our results showed that gp15 protein is able to produce a hexamer and this structure serves as a template for the assembly of gp16. The structure of gp15 alone and in the complex with gp16 was analyzed by cryo-EM at around 3.6 Å resolution. Our results suggest that gp16 could play a main role in the folding of gp15. The complex formed a 200 Å tube-like structure as it would be expected according to the proposed functional hypothesis.

S1.3 The light scattering toolkit for characterization of proteins and other bio-macromolecules

LIONEL ROUZIC,[§]

Wyatt Technology France, Toulouse, FR.

[§]lrouzic@wyatt.com

A presentation on light scattering and separation techniques for the characterization in terms of absolute molar mass and size (Rh or Rg) of proteins, other bio-macromolecules but also polymers and nanoparticles.

S1.4 Thermodynamics and protein dynamics relation in a psychrophile protein

ERNESTO ROMAN,^{1,§} Anwar Iqbal,² Javier Santos,¹ Lucas de Felipe,⁴ Fabio Almeida,² Rodolfo Martín González-Lebrero.³

¹Instituto de Química y Fiscoquímica Biológicas, UBA-CONICET, Buenos Aires, Argentina; ²Universidade Federal de Rio de Janeiro, Rio de Janeiro, Brazil; ³Instituto de Química y Fiscoquímica Biológicas, CABA, Argentina; ⁴Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, UBA, Buenos Aires, Argentina.

§ernest.roman@gmail.com

Proteins from a family share certain degree of sequence identity and structural conservation, however thermodynamic and kinetic characteristics are subtle coded. Frataxin proteins are around 7-10 kcal/mol stable but depending on the variant particular signatures such as strong modulation of stability by pH. Here we analyzed the correlation of thermodynamic stability and different-timescales dynamics as function of pH. Previous results showed that protein folds exchanging 2 protons and that pH stabilization comes mainly from entropic contributions. Titration experiments followed by NMR showed that pKa of ASP and GLU residues from loop 1 are shifted with respect to their reference value. It was also found by CPMG and relaxation experiments that small shifts in pH changes the entire dynamics from fast to slow motions (us to ms) and line broadening (seconds). Together with the chemical shift perturbation and intensity changes, the existence of another conformational states under these conditions is suggested and supported with the help of NOESY cross peaks and H-15N HSQCs at pHs around 8. We concluded that protonation and dynamics are linked and related to stability, and might be linked this frataxin's psychrophilic nature. We discuss how subtle changes in this protein makes it tunable by pH.

S1.5 Regulation of fmn biosynthesis in homo sapiens: kinetics and thermodynamics of the riboflavin kinase activity

ERNESTO ANOZ-CARBONELL,^{§,§} María Sebastián, Adrián Velázquez-Campoy, José Antonio Aínsa, Milagros Medina.

Universidad de Zaragoza, Instituto de Biocomputación y Física de los Sistemas Complejos, Zaragoza, Spain.

§eanoz@unizar.es, §[SBE bursary](#)

The human riboflavin kinase (*Hs*RFK) exhibits ATP:riboflavin kinase activity (RFK) and, therefore, catalyzes the biosynthesis of the essential cofactor flavin mononucleotide (FMN) using as substrates Vitamin B2 and ATP [1,2]. FMN is in addition the precursor of the flavin adenine dinucleotide (FAD), and together these two flavin cofactors are essential in the cellular metabolism because they function as cofactors of a plethora of flavoproteins and flavoenzymes [3]. Hence, FMN production should be thoroughly regulated to maintain the cellular and flavoproteome homeostasis. In our group, we have already characterized the RFK catalytic mechanism occurring at the c-terminal modules of different prokaryotic bifunctional fad synthetases (fads) [4], enzymes exhibiting sequence and structural homology with the eukaryotic monofunctional counterparts as *Hs*RFK. Herein, we focus on the regulation of the fmn synthesis in *homo sapiens* through the kinetic and thermodynamic characterization of the interactions of *Hs*RFK with its substrates and products, in the context of the available structural information.

References

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4. Sebastián, M., et al. *Sci. Rep.* (2017) 7: 7281.

S1.6 Subversion of endosomal sorting machinery by legionella pneumophila

ADRIANA L ROJAS-CARDONA,^{1,§} Miguel Romano-Moreno,¹ Chad D. Williamson,² David C. Gershlick,² Maria Lucas,¹ Michail N. Isupov,³ Juan S. Bonifacino,² Matthias P. Machner,⁴ Aitor Hierro.¹

¹Structural Biology Unit, Centro de Investigación Cooperativa en Biociencias - CICbioGUNE, Derio, Spain;

²Cell Biology and Neurobiology Branch, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, MD, USA; ³The Henry Wellcome Building for Biocatalysis, Biosciences, University of Exeter, Exeter, United Kingdom; ⁴Division of Molecular and Cellular Biology, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, MD, USA.

§arojas@cicbiogune.es

Microbial pathogens employ sophisticated virulence strategies to cause infections in humans. The intracellular pathogen *Legionella pneumophila* encodes RidL to hijack retromer, one of the major sorting machineries on endosomes. Using a combination of X-ray crystallography, SAXS, quantitative binding and cell-based functional assays we have solved the first structure of a bacterial effector bound to retromer, and revealed an unexpected mechanism of molecular mimicry, thus representing a new coercion strategy of retromer function.

S1.7 Transmembrane but not soluble helices fold far inside the ribosome tunnel

MANUEL BAÑÓ-POLO,^{1,§} Silvia Tamborero,¹ Carlos Baeza,¹ Anthony Hazel,² IngMarie Nilsson,⁴ Paul Whitley,³ James C. Gumbart,² Gunnar von Heijne,⁴ Ismael Mingarro.¹

¹University of Valencia, Valencia, Spain; ²Georgia Institute of Technology, Atlanta, GA, USA; ³University of Bath, Bath, United Kingdom; ⁴Stockholm University, Stockholm, Sweden.

§manuel.bano@uv.es

Integral membrane proteins are assembled into the ER membrane via a continuous ribosome-translocon channel. Once there, the hydrophobicity and thickness of the hydrocarbon core of the membrane bilayer leads to the expectation that transmembrane (TM) segments minimize the cost of harbouring a polar polypeptide backbone by engaging their polar (carbonyl and amide) groups in a regular pattern of hydrogen bonds to adopt an α -helical conformation prior to integration. Co-translational folding of tethered nascent chains into an α -helical conformation in the ribosomal tunnel has been demonstrated previously, but the features governing this folding are not well understood. In particular, little is known about what features of a helical sequence influence the propensity to acquire an α -helical structure whilst still in the ribosome. Using in vitro translation of truncated nascent chains trapped within the ribosome tunnel and molecular dynamics simulations, we show that folding in the ribosome is attained for TM helices but not for soluble helices, presumably facilitating SRP (signal recognition particle) recognition and/or a favourable conformation for membrane integration upon entering the translocon.

S1.8 Biophysical characterization of binding-induced conformational changes using switchSENSE® technology

HANNA MUELLER-LANDAU,[§] Friederike Moeller, Wolfgang Kaiser, Ulrich Rant.

Dynamic Biosensors GmbH, Martinsried/Planegg, Germany.

[§]mueller-landau@dynamic-biosensors.com

Molecular interactions and protein function crucially depend on protein structure and often involve conformational changes. switchSENSE provides a unique platform for the analysis of conformational changes in proteins by combining high sensitivity kinetics with structural analysis. Proteins are immobilized on gold microelectrodes on a biochip via electro-switchable DNA nanolevers using generic conjugation methods. The DNA nanolevers are actuated by alternating electric fields and are used to sway the proteins through the solution close to the surface at high-frequencies. The speed of the electrically actuated DNA nanolevers depends on their hydrodynamic drag and can be converted to the protein's Stokes diameter. Real-time monitoring of the switching speed allows to resolve binding kinetics and to detect structural effects, such as conformational changes or multi-monomerizations and thus provides quantitative and easy-to-interpret means for the analysis of protein size and shape. Typically, the conformation signal is clearly distinguishable from a pure binding event, which allows the simultaneous screening for binding and structural changes.

S1.9 De novo design of protein structures for binding small-molecules and nucleosomal DNA

ENRIQUE MARCOS BENTEIO,^{1,§} Benjamin Basanta,² Tamuka Chidyausiku,² Mireia Labrador,¹ Isabelle Brun-Heath,¹ Gaetano Montelione,³ David Baker,² Modesto Orozco.¹

¹*Institute for Research in Biomedicine (IRB Barcelona), The Barcelona Institute of Science and Technology, Barcelona, Spain;* ²*Institute for Protein Design, University of Washington, Seattle, WA, USA;* ³*Department of Biochemistry and Molecular Biology, The State University of New Jersey, Piscataway, NJ, USA.*

[§]emarcos82@gmail.com

Current strategies to engineer proteins for biocatalysis, molecule biosensing or therapeutics, rely on finding existing proteins having already a similar function or, at least, a suitable geometry and enough stability to tolerate mutations to achieve the new function. This dependence on existing protein structures can be a limitation for certain applications and, instead, computationally designing proteins, *de novo*, with custom-made structures should be more effective. Here we have used Rosetta to develop two *de novo* computational design approaches to tackle different biotechnological problems. For novel custom-made small-molecule binders and enzymes, we have developed a computational approach to *de novo* design thermostable protein folds with cavities formed by curved β -sheets, which were validated with NMR and X-ray crystallography [1]. For novel chromatin-based drugs and research, we have computationally designed helical bundles and TAL-like proteins to target the exposed face of nucleosomal DNA with specificity. As no structural information is available for such specific nucleosomal DNA binding, we have *de novo* designed protein-nucleosome interfaces that have not been observed in nature yet.

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S1.10 Zika virus capsid protein binds host lipid droplets via an auto-inhibitory mechanism

IVO MARTINS,[§] Ana de Souto-Martins, André F. Faustino, André Nascimento, Nuno C. Santos.

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.

[§]ivomartins@fm.ul.pt

Zika (ZIKV) and Dengue (DENV) are closely related flaviviruses, with similar capsid (C) proteins. These C proteins are mostly alpha-helical, positively charged and possess an N-terminal intrinsically disordered protein (IDP) region. The IDP region mediates DENV C binding to host lipid droplets (LDs), an interaction essential for viral replication, previously studied by us. Given ZIKV similarities to DENV, we investigated ZIKV C-LDs binding. The binding occurs, requiring specific proteins of LDs surface, as we previously reported for DENV C-LDs interaction. To further study the N-terminal IDP domain, we modeled ZIKV C full-length structure out of its sequence, resulting in 5 possible conformers. As ZIKV C is dimeric, conformers that do not allow dimerization were rejected. The two remaining conformers were arranged within the dimer, resulting in 3 possible C protein structures: one in which the IDP fully closes the access to a hydrophobic region that in DENV C is involved in the binding to LDs, another partially closed and one in which the hydrophobic core is accessible to the solvent. Thus, this suggests a mechanism of alternating and auto-inhibitory full-length ZIKV C structures, explaining their interaction with LDs, which may now be targeted in future drug development approaches.

S2 | Membrane Structure and Function

Hall Lecture Hall

Chair 1 MANUEL PRIETO

Chair 2 JESÚS SALGADO

Date: Thursday, June 21st

Time: 09:00–11:50

Sponsored by: Elsevier / CPL

S2.1 Exploring the membrane activity of a designed antimicrobial peptide with fusogenic properties

MARCIN MAKOWSKI,^{1,§,\$} Mário R. Felício,¹ Octávio L. Franco,² Sónia Gonçalves,¹ Manuel Melo,³ Nuno C. Santos.¹

¹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; ²S-inova Biotech, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco, Campo Grande, MS, Brazil; ³Instituto de Tecnologia Química e Biológica Antonio Xavier, Universidade Nova de Lisboa, Oeiras, Portugal.

§marcin.makowskimad@gmail.com, \$Best poster prize, SPBf bursary

The spread of antimicrobial resistance among bacterial pathogens is among the major threats to public health. Antimicrobial peptides (AMPs) are interesting candidates to fight bacterial infections, as their membranolytic mechanism of action is unlikely to elicit resistance. We have studied the membrane activity and selectivity of *EcAMP1R2*, a designed AMP highly specific towards *Escherichia coli*. As model membrane systems, we have used large unilamellar vesicles of different lipid compositions, including mimics of the outer (OML) and inner (IML) membranes of *E. coli*, and actual *E. coli* cells. Using a combination of fluorescence spectroscopy, flow cytometry and light scattering spectroscopy, we have found that *EcAMP1R2* discriminates between mammalian and bacterial-like membranes. The peptide causes hyperpolarization of *E. coli* cells, indicating inability for pore formation. Moreover, *EcAMP1R2* promotes the (hemi)-fusion of IML vesicles, without neutralizing its surface potential. This suggests that the interaction of *EcAMP1R2* with IML vesicles results in major membrane lipid rearrangements able to alter the membrane curvature. We propose that the peptide induces cardiolipin demixing at the plasmatic membrane. Molecular dynamics simulations with the Martini force field were performed to test the validity of our hypothesis. Simulations indicated dimer formation on the membrane, which prompted experimental validation by NMR.

S2.2 Biophysical experiments and molecular dynamics simulation of self-assembling cyclic peptide nanotubes at biological model membranes

BARBARA CLARO,^{1,§,§} Antonio Peon,¹ Eva Freire,² Rebeca García-Fandino,¹ Margarida Bastos.¹

¹CIQ-UP Dep. Chemistry and Biochemistry, FCUP, Porto, Portugal; ²CIQUS Singular Research Centre in Chemical Biology and Molecular Materials, USC, Santiago de Compostela, Spain.

§barbaramariaclaro@gmail.com, §*EBSA bursary*

The continuous growth of resistance to conventional antibiotics [1] leads to an urgent need of new classes of antimicrobial agents with alternative mechanisms of action that do not easily induce resistance, i.e., new antibiotic paradigms. One approach involves the use of intelligent materials that only form the active species only upon contact with the bacterial membrane, such as SCPNs (Self-assembling Cyclic Peptide Nanotubes). They are currently seen good antimicrobial candidates due to their robust secondary structure and high activity [2]. We used biophysical experimental techniques (DSC, ITC, SAX, DLS, ATR-FTIR) together with an in-silico approach to study the interaction of some antimicrobial SCPNs with model membranes with the goal of characterizing their interactions, leading to the unveiling of their possible mechanism of action. Preliminary Molecular Dynamics (MD) studies with different force-fields, and at atomistic and coarse-grained resolution for different model membranes will be shown and compared with results from DSC experiments. The results from SAXS and ATR-FTIR provide information on the structural features of these systems.

S2.3 Free-standing membranes inside microfluidic devices and their use for the study of membrane nanotubes

AURORA DOLS-PÉREZ,[§] Roland Kieffer, Victor Marín, Marie Eve Aubin-Tam.

Department of Bionanoscience, Kavli Institute of Nanoscience, Delft University of Technology, Delft, The Netherlands.

§a.dolsperez@tudelft.nl

Membranes and lipid nanotubes play a vital structural role in different cellular organelles such as the endoplasmic reticulum, mitochondria and Golgi apparatus, but also in communication processes such as inter and intracellular exchanges and cellular migration. Their study is often carried on vesicles, supported lipid bilayers or living cells. In these approaches, it is challenging to achieve asymmetric lipid distribution, dynamic buffer control and zero curvature. In contrast, we show that the use of a freestanding lipid bilayer on a chip solve these limitations and present additional advantages such as easy access to both sides of the membrane, compatibility with electrical measurements, formation of multiple membranes in the same device, possibility to create asymmetric membranes, possibility to circulate different solutions, and full compatibility with optical techniques. To form the bilayers inside the devices, we flow by parallel channels an organic solvent containing lipids followed by an aqueous solution. The membrane will assemble by the contact of the lipid interfaces over the apertures connecting the channels. This method of lipid bilayer formation combined with optical tweezers have allowed us to solve the previously mentioned limitations. Furthermore, our approach enables to form nanotubes with the highest efficiency and to create nanotubes longer than 250 μm .

S2.4 Single cysteine mutants of actinoporins sticholysins i and ii as tools for the study of membrane heteropores assembly

ESPERANZA RIVERA-DE-TORRE,^{§,§} Sara García-Linares, Juan Palacios-Ortega, José G. Gavilanes, Álvaro Martínez-del-Pozo.

Departamento de Bioquímica y Biología Molecular, Facultad de Ciencias Químicas, Universidad Complutense de Madrid, Madrid, Spain.

[§]esperanza.rivera.dotorre@gmail.com, [§]*SBE bursary*

Actinoporins main components of many sea anemones venom. They are α -pore-forming-toxins, remaining stably folded in solution until encountering sphingomyelin-containing lipidic membranes. Then, they become oligomeric integral membrane structures forming a pore. Actinoporins appear as multigene families: a single anemone displays genes coding for similar toxins with different cytolytic power. *Stichodactyla helianthus* produces at least two different actinoporins, sticholysin I and II, with 93% of sequence identity but different hemolytic activity. However, they act synergically, supporting a complex cytolytic regulation based on the formation of heteropores. An observation which transcends the specific field of these proteins and should be considered as a general feature of pore-forming toxins, given their multigene character. In order to explore the molecular details of this synergy, their specific interactions, and the heteropores stoichiometry, we take advantage of their cysteinless character and use site-directed mutagenesis to introduce single Cys residues at their potential surfaces of interaction. These free SH groups are used to specifically label the proteins with maleimide-derivative fluorescence probes. The labelled mutants are employed in experiments of fluorescence resonance energy transfer measurements. The results obtained are discussed in terms of the molecular details involving the interactions needed to explain pore-formation and the observed synergic modulation.

S2.5 Bringing disorder to order: new insights into the structure/function correlation of the golgi reassembly and stacking protein

ANTÔNIO JOSÉ DA COSTA-FILHO,^{§,§}

University of São Paulo, São Paulo, Brazil.

[§]ajcosta@usp.br, [§]*Sponsored by ISMAR*

Golgi Reassembly and Stacking Proteins (GRASPs) are involved in cell processes that seem paradoxical: responsible for shaping the Golgi cisternae and also participating in unconventional secretion mechanisms that bypass the Golgi. The exact molecular mechanisms underlying each process remain elusive. GRASP structures are constituted by the so-called GRASP domain and by the SPR domain, which is rich in serine and proline, and detailed structural information has been limited to the GRASP domain only. In this talk, we will show results obtained from full-length GRASPs, which unravel an unexpected structural feature: the presence of intrinsically disordered regions, more specifically, GRASP is in a molten globule state without the need of any mild denaturing condition. Moreover, GRASP undergoes a disorder-to-order transition in the presence of mimetics of cell conditions. Changes in the dielectric constant, such as those experienced close to the membrane surface, seem to be the major factor in inducing that transition and also in leading to the formation of amyloid-like fibrils in the *S. cerevisiae* GRASP. We propose a model on how the cell could use the GRASP sensitivity to changes in the dielectric constant as well as its fibril formation propensity during different cell-cycle periods.

S2.6 The impact of atypical sphingolipids on the biophysical properties of model and cell membranes

TANIA CUNHA BRANCO-SANTOS,^{1,§,§} Patricia Bastiao,² Essa Saied,³ Manuel Prieto,¹ Christoph Arenz,² Thorsten Hornemann,⁴ Liana Silva.²

¹*iMed.Ulisboa – Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal;* ²*CQFM-IN and IBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal;* ³*Institute for Clinical Chemistry, University Hospital Zurich, Zurich, Switzerland;* ⁴*Humboldt Universität zu Berlin, Institute for Chemistry, Berlin, Germany.*

§tsantos.cb@gmail.com, § *SPBf bursary*

Sphingolipids (SLs) participate in many cellular events. Their building blocks, long chain sphingoid bases, are formed from L-serine and palmitoyl-CoA. However, alanine and glycine can also be used, forming atypical 1-deoxy-sphingoid bases that lack the OH at the C1 position. Elevated levels of 1-deoxySLs are associated with the development of HSAN1 and diabetes type II. Nevertheless, their biological significance and the molecular mechanisms underlying their pathological role remain elusive. Using fluorescence-based methodologies we showed that, in contrast to their canonical counterparts, 1-deoxySLs failed to form highly-ordered gel domains in fluid model membranes. Moreover, elevated cellular levels of 1-deoxySLs increased the overall membrane fluidity compared to control cells. To investigate if this was a consequence of impaired H-bond network due to the lack of the C1-OH group, the biophysical properties of 1-methoxy-SLs were studied. The ability to form gel domains and decrease membrane fluidity was reestablished, although to a less extent. These results indicate that C1 headgroup of the SLs determines the formation of tightly packed domains. In conclusion, canonical and 1-deoxySLs lead to opposite changes in membrane biophysical properties, suggesting a possible mechanism to mediate the distinct biological actions of these species.

S2.7 Siramesine's anticancer activity: a biophysical approach

JOSÉ LOPES-DE-ARAÚJO,^{1,2,§,§} Clara Fonte,¹ Diana Felícia Carvalho,¹ Cláudia Nunes,¹ Salette Reis.¹

¹*LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal;* ²*Instituto Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.*

§jose.p.l.araujo@gmail.com, § *SPBf bursary*

Siramesine is a cationic amphiphilic drug that induces cell death displaying a promising anticancer activity in *in vitro* and *in vivo* studies. Nevertheless, the specific mechanisms by which Siramesine causes cell death are not well understood. Some authors suggest that they may be related with the interaction of Siramesine with specific cell organelles, like the mitochondria (loss of mitochondrial membrane potential, production of ROS and cytochrome C release) and the lysosome (lysosomal leakage and production of ROS). It has been related that Siramesine can interact with a negative lipid, the phosphatidic acid. So, it can also possibly interact with other lipids that compose the membranes, specially the negatively charged phospholipids. Therefore, we hypothesize that the rapid cell death accompanied by loss of cellular integrity may be due to the interaction of Siramesine with the phospholipids of specific membranes. To study these interactions, we applied different biophysical techniques to different membrane models mimicking distinct cell membranes. The obtained results suggest that Siramesine interacts in a concentration-dependent manner with the studied models.

Financial support: Authors thank the financial support of National Funds from FCT and FEDER under Program PT2020 (project007265-UID/QUI/50006/2013). This work was supported by POCH, FCT(IF/00293/2015) and FCT PhD Programmes (BiotechHealth Programme. PD/BD/114012/2015).

S2.8 In vitro assessment of licofelone-membrane interactions: Insights into drug development?

CATARINA PEREIRA-LEITE,^{1,2,§,§} Cláudia Nunes,¹ Ana Coutinho,^{3,4} Joana C. Ricardo,³ Aleksander Fedorov,³ Manuel Prieto,³ José C. Bozelli Jr.,^{2,5} Shirley Schreier,² Iolanda M. Cucovia,² Salette Reis.¹

¹ICETA, LAQV, REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Portugal; ²Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, Brazil; ³Centro de Química-Física Molecular and Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Portugal; ⁴Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Portugal; ⁵Department of Biochemistry and Biomedical Sciences, McMaster University, Canada.

§ catarinapl12@gmail.com, § SPBf bursary

Since the in vitro assessment of drug-membrane interactions has been providing valuable information about the therapeutic and toxic actions of commercialized drugs [1], the aim of this study is to highlight the relevance of studying drug-membrane interactions to drug development. In this work, the interactions of licofelone, a dual-acting compound under development, with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers at physiological conditions were analyzed combining various biophysical techniques. Drug partitioning was evaluated by derivative spectrophotometry and drug effects on the biophysical properties of the DMPC bilayer was assessed by differential scanning calorimetry, electron paramagnetic resonance, fluorescence anisotropy and X-ray diffraction measurements. Licofelone was found to have high affinity to DMPC bilayers, to decrease the temperature and cooperativity of the lipid phase transition, and to induce changes in the packing and ordering of DMPC acyl chains. Both therapeutic and toxic effects may arise in vivo from the licofelone-induced alterations in the organization of phospholipid bilayers. Therefore, the preclinical characterization of drug-membrane interactions can be valuable to obtain a complete description of the pharmacological actions of novel drugs.

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References

1. Pereira-Leite C., et al. *Prog. Lipid Res.* (2013) 52: 571.

S3 | Channels, Receptors and Transporters

Hall Auditorium

Chair 1 JOÃO MORAIS-CABRAL

Chair 2 FRANCISCO BARROS

Date: Thursday, June 21st

Time: 15:00–18:00

Sponsored by: The Spanish Ion Channel Initiative (SICI)

S3.1 Molecular function of $\alpha 7$ nicotinic receptors

CECILIA BOUZAT.[§]

INIBIBB-UNS/CONICET-Argentina.

[§]inbouzat@criba.edu.ar

The $\alpha 7$ nicotinic receptor is expressed in brain and non-neuronal cells. Enhancement of $\alpha 7$ activity by positive allosteric modulators (PAMs) is emerging as a therapeutic strategy for cognitive and inflammatory disorders. We have focused on understanding $\alpha 7$ function and potentiation. We revealed that PAMs enhance $\alpha 7$ activation by increasing the open-channel lifetime and inducing prolonged activation episodes. Although $\alpha 7$ has been considered the homomeric member of the family, a novel $\alpha 7\beta 2$ receptor has been recently discovered in human brain. We generated $\alpha 7\beta 2$ receptors with fixed stoichiometry by two approaches comprising concatenated and unlinked subunits. We found that $\beta 2$ can assemble with $\alpha 7$ subunits resulting in receptors with different stoichiometries, kinetic signatures and PAM selectivity. This information provides fundamental basis required to decipher the role of $\alpha 7\beta 2$ in native cells. In humans, there is a truncated $\alpha 7$ subunit (*dup $\alpha 7$*) that lacks part of the ACh-binding site and results from a partial duplication of the $\alpha 7$ gene. Its role remains unknown. We demonstrated that *dup $\alpha 7$* acts as a negative modulator, cannot form channels, but can assemble with $\alpha 7$ into functional heteromeric receptors. Deciphering the molecular basis underlying $\alpha 7$ responses has implications for the design of novel therapeutic compounds.

S3.2 Fine-tuning of store-operated Ca^{2+} entry by SARAF and EFHB

JUAN ANTONIO ROSADO,[§]

Universidad de Extremadura, Cáceres, Spain.

[§]jarosado@unex.es

Store-operated Ca^{2+} entry (SOCE), a mechanism regulated by the filling state of the intracellular Ca^{2+} stores, is an apparently ubiquitous and functionally relevant mechanism for Ca^{2+} influx. The endoplasmic reticulum (ER) Ca^{2+} sensor, STIM1, and the plasma membrane (PM) Ca^{2+} channel, Orai1, are the key components of SOCE. Regulation of Ca^{2+} entry through store-operated channels is essential to maintain an appropriate intracellular Ca^{2+} homeostasis and prevent cell damage. SARAF is a single transmembrane segment-containing protein that modulates STIM1 by preventing spontaneous SOCE activation and mediating slow Ca^{2+} -dependent inactivation (SCDI) of Orai1 channels. At resting conditions, SARAF is associated with STIM1, preventing its activation, but upon Ca^{2+} store depletion SARAF initially dissociates from STIM1, facilitating that STIM1 interacts with and activates Orai1, and, after a few seconds, SARAF re-interacts with STIM1 to promote SCDI. We have recently identified EFHB (EF-hand domain family member B) as a widely expressed modulator of SOCE that mediates the dynamic interaction of SARAF with STIM1; thus modulating the STIM1-Orai1 interaction and, subsequently, SOCE. Summarizing SARAF and EFHB fine-tune SOCE, shaping Ca^{2+} signals and preventing Ca^{2+} overload.

Financial support: Supported by MINECO (BFU2016-74932-C2-1-P) and Junta de Extremadura-FEDER (IB16046 and GR15029).

S3.3 Analysis of anthrax toxin channel current fluctuational dynamics

EKATERINA NESTOROVICH,[§] Goli Yamini.

The Catholic University of America, Washington, DC, USA.

[§]nestorovich@cua.edu

Nature's most potent intracellularly active exotoxins are secreted with their own delivery components, which assist them in trespassing the membrane barrier. Anthrax toxin, the etiological agent of *Bacillus anthracis*, is a tripartite exotoxin composed of lethal factor and edema factor, that act intracellularly, and PA_{63} , that forms channels to mediate the lethal and edema factors translocation. The cryo-EM imaging of PA_{63} revealed a stable 'frozen flower on a stem' structure. The reality is more complex. In bilayers, PA_{63} exhibits different modes of fluctuational behavior, including $1/f$ noise, manifested by fast flickering between the open and closed states. Notably, identical fluctuations are observed in the clostridial binary toxin channels, which share functional similarities with PA_{63} . However, the $1/f$ closures remained undetected in F427A mutant, where the 'hydrophobic belt' (ϕ -clamp), critical for protein translocation, is removed. Here, we discuss the feasibility of several models for the $1/f$ noise. Our recent findings, including PEG partitioning experiments, suggest that the $1/f$ noise in PA_{63} occurs as a result of 'hydrophobic gating' at the ϕ -clamp region; the phenomenon earlier observed in many water-filled channels 'fastened' inside by hydrophobic belts. This finding can elucidate the functional role of $1/f$ noise in the enzymatic factor translocation.

S3.4 Structures of gpcr-g protein complexes by volta phase plate electron cryo-microscopy

JAVIER GARCÍA-NAFRÍA,[§] Rony Nehme, Yang Lee, Christopher G. Tate.

MRC LMB, Cambridge, United Kingdom.

[§]jgarcia@mrc-lmb.cam.ac.uk

G-protein coupled receptors (GPCRs) are the largest family of membrane receptors in the human body and are a target for ~40% of the currently approved FDA drugs. GPCRs sense a variety of molecules (lipids, hormones, neurotransmitters... etc) and transduce the signal to the intracellular milieu by coupling to and activating heterotrimeric G-proteins. There are four families of G-proteins (G_S , $G_{I/O}$, G_q , $G_{12/13}$), each one activating or inhibiting a signalling pathway in the cell. Only information is available for how G_S type of G-proteins couple to GPCRs. In order to understand the mechanisms of function of this family we have optimized structural determination of GPCRs by cryo-electron microscopy. We first determined the active state of the adenosine A_{2A} receptor complexed with a heterotrimeric G-protein (G_S). The Volta phase plate (VPP) was essential to attain 'side-chain' resolution. After the proof-of-concept, we determined the structure of the active state of the serotonin $5HT_{1B}$ receptor bound to a new G_O heterotrimer. The structure describes the activation mechanism of this receptor and starts to tackle one of the main questions in the field, which is, with ~800 GPCRs in the human body and only four families of G-proteins, how GPCRs select for their specific G-protein.

S3.5 Anion conduction and gating in the calcium-activated chloride-channel TMEM16a

CRISTINA PAULINO,[§]

University of Groningen, Groningen, The Netherlands.

[§]c.paulino@rug.nl

The TMEM16 family of eukaryotic membrane proteins plays a key role in a variety of physiological processes. Despite their close relationship, TMEM16 members feature a striking functional diversity, as some work as calcium-activated anion channels and others as lipid scramblases. Although the structure of the nhTMEM16 scramblase revealed the general architecture of the protein family it remained unclear how a channel has adapted to cope with its distinct functional properties. We have addressed this question by determining the cryo-EM structure of the mouse TMEM16A channel in presence and absence of calcium. The protein shows a similar organization to nhTMEM16, except for changes at the site of catalysis. Here, the conformation of transmembrane helices, which line a membrane-spanning furrow in scramblases, has changed to form an enclosed aqueous pore that is largely shielded from the membrane. Pronounced differences between the calcium-bound and calcium-free mTMEM16A structures are confined to the inner half of TMH6, which acts a gating element during activation. Strikingly, calcium ions not only regulates the channel in a conventional allosteric manner, by narrowing the pore neck, but also by directly introducing an electrostatic barrier at the pore entrance.

S3.6 Modulation of the KcsA potassium channel by anionic phospholipids: role of the non-annular arginines

JOSÉ MANUEL GONZÁLEZ-ROS,^{1,§} José Antonio Poveda-Larrosa,¹ A. Marcela Giudicci,² M. Lourdes Renart,² Óscar Millet,³ Andrés Morales-Calderón,⁴ Victoria Oakes,⁵ Carmen Domene.⁵

¹UMH-IBMC, Spain; ²UMH-JMGR, Spain; ³Biogune, Bilbao, Spain; ⁴Univ. Alicante, Alicante Spain; ⁵Univ. Bath, Bath, United Kingdom.

§gonzalez.ros@umh.es

The role of two arginine residues R64 and R89 present at the non-annular lipid binding sites of KcsA on the modulation of channel activity by anionic lipids, has been investigated. We have worked with the wild-type KcsA and with different arginine mutant channels reconstituted into membranes containing increasing amounts of the anionic lipid PA (phosphatidic acid). The characterization of these samples using a combined molecular biology, electrophysiology, NMR spectroscopy and molecular dynamics (MD) approach, allows to propose a molecular mechanism encompassing the whole modulatory process, from the binding of anionic lipids to the non-annular sites, to the downstream sequence of events leading to changes in the conformation and properties of the channel inactivation triad and selectivity filter, which finally determine the functional state of the channel.

S3.7 Structure of the cNMP domain of the magnesium transport mediator CNNM4.

PAULA GIMÉNEZ-MASCARELL,^{1,§} Serge Hardy,² Tilman Breiderhoff,³ Antonio Díaz-Quintana,⁴ Irene Díaz-Moreno,⁴ Francisco Blanco,¹ Dritan Siliqi,⁵ Michel L. Tremblay,² Dominik Müller,³ Luis Alfonso Martínez-Cruz.¹

¹Structural Biology Unit, CIC bioGUNE; ²Department of Biochemistry and Goodman Cancer Research Centre, McGill University, USA; ³Department of Pediatric gastroenterology, nephrology and Metabolic disorders. Charité Universitätsmedizin; ⁴IIQ, cicCartuja, Universidad de Sevilla and CSIC, Sevilla, Spain; ⁵Istituto di Cristallografia, Consiglio Nazionale delle Ricerche (CNR), Bari, Italy.

§pgimenez@cicbiogune.es

Magnesium (Mg^{2+}), is the second most abundant intracellular cation in cells, playing different functions as neurotransmission, proliferation and enzyme activity. Its homeostasis is tightly controlled by different proteins, among which are the four members of the cyclin M family (CNNM1-4). These proteins are ubiquitously distributed and mediate magnesium transport throughout the cell membranes. CNNMs are structurally complex and are formed by an N-terminal domain, followed by a transmembrane region and two intracellular modules. In 2014 we found that the first intracellular domain of CNNM2, also known Bateman module, forms stable dimers that are subject to conformational changes upon binding of MgATP. (Corral-Rodriguez et al. 2014, Giménez-Mascarell et al. 2016). Here we present, the crystal structure of the second intracellular domain of CNNM4, which structurally belongs to the family of cyclic nucleotide binding-like domains (cNMPs), which are present, among others, in ionic channels such as the KCNH family of K^+ transporters. Our data reveal not only the oligomeric state of this module, but the intrinsic chemical-physical features of the potential cyclic-nucleotide binding site, thus providing a three-dimensional template that may help decipher the structural basis underlying magnesium homeostasis in mammals.

S3.8 Exploring potassium channels selectivity and inactivation through steady-state and time-resolved fluorescence spectroscopy

MARÍA LOURDES RENART-PÉREZ,^{1,§} Estefanía Montoya,² Ana Marcela Giudicci,² José Antonio Poveda-Larrosa,² José Manuel González-Ros,² Manuel Prieto,¹ Ana Coutinho.¹

¹*CQFM and IN - Institute of Nanosciences and Nanotechnology, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal;* ²*Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Elche (Alicante) Spain.*

§ lrenart@umh.es

Potassium channels selectivity, permeation and inactivation properties are exquisitely executed by the selectivity filter (SF) domain and its allosteric interplay with other protein structures, such as the C-terminal segment and pore helix. Here we use the prokaryotic potassium channel KcsA to explore how different channel conformations (closed/open; conductive/collapsed/inactivated) could account for its ion conduction characteristics. Steady-state and time-resolved fluorescence spectroscopy approaches were used to characterize ion-protein association equilibria and SF/pore helix changes according to type/concentration of cations and pH gating. Our results indicate that in the closed state, the SF is asymmetric in terms of cation binding and when its conductive conformation is stabilized, there is a marked decrease in Na⁺ affinity. These selective binding and selective exclusion seems to partially account for K⁺/Na⁺ selectivity. On the other hand, novel analysis of the homo-FRET process in W67 single Trp mutant channel allowed us to measure pore helix intersubunit distances. Interestingly, these time-resolved fluorescence experiments revealed that SF collapsed (low [K⁺], pH 7) and inactivated (high [K⁺], pH 4) states are not structurally equivalent and that the occupation of S2 binding site is relevant to stabilize this last conformation.

Financial support: BFU2012-31359 and BFU2015-66612-P (MINECO/FEDER, Spain); FAPESP/20107/2014 (FCT, Portugal).

S4 | Cellular Biophysics

Hall Lecture Hall

Chair 1 TERESA GIRÁLDEZ

Chair 2 GRAÇA SOVERAL

Date: Thursday, June 21st

Time: 15:00–18:00

S4.1 Unravelling the activity and mechanism of action of pepR, a viral-derived membrane-active peptide, against *Staphylococcus aureus* biofilms

ANA SALOMÉ VEIGA,^{1,§} Sandra N. Pinto,² Susana A. Dias,¹ Dalila Mil-Homens,³ Fábio Fernandes,² Javier Valle,⁴ David Andreu,⁴ Manuel Prieto,² Miguel Castanho,¹ Ana Coutinho.²

¹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; ²Centro de Química-Física Molecular e IN, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal; ³iBB-Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal; ⁴Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain.

§ aveiga@medicina.ulisboa.pt

Infections caused by bacterial biofilms are a major healthcare problem given their reduced susceptibility to conventional antibiotics. Thus, it is urgent to develop new and effective antimicrobial agents against bacterial biofilms. Antimicrobial peptides (AMPs) have been considered potential alternatives for this purpose. In this work we show that pepR, a multifunctional peptide derived from the Dengue virus capsid protein, is able to both prevent the formation and act on preformed *Staphylococcus aureus* biofilms. Detailed mechanistic studies using flow cytometry and confocal fluorescence microscopy show that pepR targets the bacterial membrane. Prevention of biofilm formation results from the fast bactericidal membrane permeabilization of bacteria in the planktonic form. More important, the peptide is able to diffuse through a preformed biofilm and rapidly kill biofilm-embedded bacteria through the same mechanism. Overall, our work demonstrates the potential of pepR as a lead towards the development of novel membrane-active antibiofilm peptides.

S4.2 Multiscale viscoelasticity of living systems

SONIA CONTERA,[§] Jacob Seifert, Zeinab al Rekabi, Alba Rosa Piacenti.

University of Oxford, Oxford, United Kingdom.

[§]sonia.antoranzcontera@physics.ox.ac.uk

The mechanical properties of biological systems have finally come to the forefront of biological research, since there is a growing realisation that these properties modulate physiological activity throughout all biological temporal and spatial scales. Much emphasis is being put on elasticity, the capacity of a material to instantaneously recover after deformation. However, very few biological materials are purely elastic; their structures have the capacity of dissipating energy, i.e. they are viscous and respond to deformations with a characteristic time scales. While elasticity is capable of propagating mechanical signals and waves, viscosity can dampen vibration and facilitate deformation. The combination of elasticity and viscosity sets the timing of biological movements and vibrations. We have developed advanced dynamic atomic force microscopy (AFM) methods to fully characterize viscoelastic properties of living cells, membranes, tissues and even single molecules using scanning probe microscopy. Our results demonstrate that cells membranes, cells walls, and whole cells in tissues *in living multicellular organisms* pattern and modulate their elasticity/viscosity ratio to adapt to their function, e.g. in growth, transport, and mechanical signal transduction.

S4.3 Changes on elasticity and morphology of erythrocytes from amyotrophic lateral sclerosis patients

CATARINA SOUSA-LOPES,^{1,§,§} Ana Catarina Pronto-Laborinho,¹ Mamede de Carvalho,¹ Nuno C. Santos,² Filomena A. Carvalho.¹

¹*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal;* ²*Serviço de Neurofisiologia, Hospital de Santa Maria, Centro Hospitalar Lisboa Norte, Lisboa, Portugal.*

[§]catarinalopes@medicina.ulisboa.pt, [§]*SPBF bursary*

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease. Patients' complications, such as venous thromboembolism (VTE), promote changes in haemodynamic properties and abnormalities in red blood cells (RBC) membrane and on its lipid content. Our main goal was to evaluate changes in the elastic and morphological properties of RBCs in ALS and compare them with the erythrocytes from healthy donors. By atomic force microscopy (AFM), RBC membrane roughness, elasticity and morphological parameters were analysed for both groups. Patients' RBCs are stiffer, have higher penetration depth and are more capable to deform, presenting an increased membrane roughness. Morphological changes on RBCs from ALS patients were also assessed by AFM, showing lower thickness and higher cell area. Zeta-potential analysis showed that the surface of patients' RBCs is less negatively charged, which may be due to a lower density of sialic acid residues. Fluorescence spectroscopy showed that RBC membranes from ALS patients are more fluid. This may be associated with changes on membrane lipid composition and packing. We conclude that ALS disease leads to significant electrostatic and morphologic changes in RBC membranes. These findings may contribute to understand the complex interplay between ALS disease progression rate and RBC lipid profile.

S4.4 Biophysical mechanisms of collagen export from the endoplasmic reticulum

FÈLIX CAMPELO-AUBARELL,^{1,§} Ishier Raote,² Maria Ortega-Bellido,² Antonio J. M. Santos,² Ombretta Foresti,² Chong Zhang,³ Maria F. García-Parajo,¹ Vivek Malhotra.²

¹ICFO-Institut de Ciències Fotoniques, Barcelona, Spain; ²CRG-Centre for Genomic Regulation, Barcelona, Spain;

³Universitat Pompeu Fabra, Barcelona, Spain.

§ felix.campelo@icfo.eu

Collagens, which constitute 25% of our dry body weight, are synthesized in the endoplasmic reticulum (ER), from where they are exported along the secretory pathway for release into the extracellular space. Collagens are however too large to fit into the standard vesicles of 60 nm average diameter that export secretory cargoes from the ER. Despite its fundamental importance, the mechanisms by which collagens are exported from the ER still remain a mystery. An ER-resident transmembrane protein, TANGO1, is required for the export of collagens by modulating and physically connecting the cytosolic membrane-remodeling machinery to the collagens in the ER lumen. To monitor the organization and dynamics of collagen export from the ER and to obtain a physical understanding of the mechanisms of TANGO1-mediated export, we employed a multidisciplinary approach that combines state-of-the-art genetic manipulations together with advanced optical techniques, such as STORM and STED nanoscopy, and biophysical modeling. Specifically, I will focus on the development and analysis of a continuous elastic model of the membrane to address how TANGO1 modulates ER membranes to generate a mega transport carrier. Altogether, our approaches start to provide a comprehensive quantitative molecular and mechanical understanding of how collagens are exported from the ER.

S4.5 Exploring myosin's roles during cytokinesis

ANA XAVIER-CARVALHO,[§] Daniel Osorio-Sampaio, Fung-Yi Chan, Joana Saramago, Joana Leite, Marta Silva.

i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal Instituto de Biologia Molecular e Celular – IBMC, Porto, Portugal.

§ anacarvalho@ibmc.up.pt

Cytokinesis is the last step of cell division that physically partitions the mother cell into two daughter cells. It is an actomyosin-dependent process and requires the assembly and constriction of an actomyosin contractile ring that is integrated in the cell acto-myosin cortex. As other actomyosin-dependent processes, cytokinesis involves global force generation and cell shape changes. Force generation has traditionally been thought to be due to myosin ability to translocate actin filaments at the expense of ATP but that idea has been challenged. We have generated *C. elegans* strains expressing myosin motor mutants and are exploring the impact of expressing these mutants in contractile ring formation and constriction during early embryonic cytokinesis.

S4.6 Meiotic nuclear oscillations are necessary to avoid excessive chromosome associations

MARIOLA CHACÓN-RODRÍGUEZ,^{1,§} Vasily Zaburdaev,² Iva M. Tolić.³

¹*Technische universität Dresden, Dresden, Germany;* ²*Max Planck Institute for the Physics of Complex Systems, Dresden, Germany;* ³*Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia.*

§mariola.chacon@tu-dresden.de

During meiosis, homologous chromosomes need to find each other and pair with high precision. Fission yeast solves this problem by folding chromosomes in loops and pulling them through the viscous nucleoplasm. Pairing of homologous chromosomes depends on nuclear oscillations. However, how nuclear oscillations help pairing is unknown. Here, we show that homologous loci typically pair when the spindle pole body is at the cell pole and the nucleus is elongated, whereas they unpair when the spindle pole body is in the cell center and the nucleus is round. Inhibition of oscillations demonstrated that movement is required for initial pairing and that prolonged association of loci leads to mis-segregation. The double-strand break marker Rec25 accumulates in elongated nuclei, indicating that prolonged chromosome stretching triggers recombinatory pathways leading to mis-segregation. Mis-segregation is rescued by overexpression of the Holliday junction resolvase Mus81, suggesting that prolonged pairing results in irresolvable recombination intermediates. We conclude that nuclear oscillations exhibit a dual role, promoting initial pairing and restricting the time of chromosome associations to ensure proper segregation.

S4.7 Antimicrobial peptides PaMAP2 and PaMAP1.9 reveal anticancer activity

MÁRIO R. FELÍCIO,^{1,§,§} Octávio L. Franco,² Nuno C. Santos,¹ Sónia Gonçalves.¹

¹*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal;* ²*S-INOVA, Programa de Pós-Graduação em Biotecnologia, Universidade Católica Dom Bosco, Mato Grosso do Sul, Campo Grande, Brazil.*

§mrfelicio@medicina.ulisboa.pt, §*SPBf bursary*

Antibiotic resistance is a major public health problem that is expected to lead pharmaceutical companies to a new paradigm, where conventional molecules will need to be replaced. As a matter of fact, the World Health Organization has already pointed out the urgency in finding new molecules against different pathogens, named the ESKAPE group (Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species). Additionally, in cancer therapy, the number of resistance cases in patients has increased, with associated infections being a cause of death in many of those cases. Antimicrobial peptides (AMPs) are considering a new promising alternative for infectious and cancer therapies. Being small, cationic and hydrophobic, their major advantage is the difficulty for pathogens to acquire resistance. We studied PaMAP2 and PaMAP1.9, two synthetic AMPs that showed effectiveness against multiresistant clinical isolated bacteria. Using biophysical techniques (fluorescence spectroscopy, flow cytometry and confocal microscopy), focused on peptide-membrane interactions, we tested if these AMPs could be efficient against cancer cells. Our data demonstrate that, besides antimicrobial activity, PaMAP1.9 (but not PaMAP2) can also target cancer cells, showing that AMPs can have a dual activity, which is important for cancer therapy patients.

S4.8 Relaxational kinetics in red blood cell mechanics: linking physical to biological aging

MARTA GIRONELLA-TORRENT,^{1,§} Fèlix Ritort.²

¹*Departament de Física de la Matèria Condensada, Facultat de Física, Universitat de Barcelona, Barcelona, Spain;*

²*Departament de Física de la Matèria Condensada, Facultat de Física, Universitat de Barcelona, Barcelona, Spain and CIBER-BBN, Instituto de Salud Carlos III, Madrid, Spain.*

§mgironella4@gmail.com

Red blood cells (RBC) are one of the most abundant and simplest cells in human body. Only composed of a lipid bilayer and an spectrin cytoskeleton, their shape, mechanics and aging are fundamental features to understand and treat the majority of blood diseases. In this project we study relaxational processes in the mechanics of RBC using optical tweezers. We use two different approaches in order to understand the viscoelastic response of the RBC: 1) Pulling experiments, where we pull and push the RBC at different maximum forces and different pulling velocities to extract information of the force-distance curves and; 2) Relaxation experiments, where we apply a force jump to the RBC and measure force relaxation. From these two kind of experiments we are able to characterize four different time-scales, three of them related to membrane-cortex interaction, the other one (which is the longest) shows a stiffening of the RBC that we hypothesize it is linked to aging in the RBC. The correlation between the time-scales allows us to globally understand the temporal evolution of RBC and link physical to biological aging.

S5 | Nucleic Acids & Supramolecular Complexes

Hall Lecture Hall

Chair 1 EURICO CABRITA

Chair 2 JOSÉ LÓPEZ-CARRASCOSA

Date: Friday, June 22nd

Time: 09:00–11:00

S5.1 Exploring the conformational plasticity of tau-tubulin complex by single-molecule fret: insights into function of a dysfunctional protein

ANA M. MELO,^{1,§} Juliana Coraor,² Garrett Alpha-Cobb,² Shana Elbaum-Garfinkle,² Elizabeth Rhoades.³

¹Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA Present address: CQFM-IN and IBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal;

²Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, CT, USA; ³Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA.

§ anammelo@tecnico.ulisboa.pt

Tau is a neuronal intrinsically disordered protein (IDP), whose aggregation is linked to several tauopathies, including Alzheimer's disease. Additionally, the loss of its native interaction with microtubules is thought to contribute for pathology. Despite intense study, structural details of the tau-tubulin complex are lacking, in part due to its highly dynamic nature and the capacity to promote tubulin polymerization. Here, we use intramolecular single-molecule Förster Resonance Energy Transfer (smFRET) to determine topological features of tau bound to soluble tubulin heterodimers. Tau adopts an overall extended conformation upon tubulin binding, in which the long-range of contacts between both termini and the microtubule binding region (MTBR) that characterize its compact solution structure are diminished. Surprisingly, the individual repeats within MTBR that directly interface with tubulin undergo an expansion in order to accommodate tubulin binding without changing the overall MTBR dimensions. Notably, it suggests the formation of such a “fuzzy complex”, in which tau displays significant flexibility to allow for local changes in conformation while preserving global features. Moreover, our results contrast differences in tau isoforms and a conformational ensemble of tubulin-bound state distinct from its aggregation-prone structure. This work draws attention to the importance of the role of tau's conformational plasticity in function.

S5.2 Fitting tools for integrative structural biology

PABLO CHACÓN,[§] Pablo del Solar, José Ramón López-Blanco.

IQFR-CSIC, Madrid, Spain.

[§]pchacon@iqfr.csic.es

Modeling cryo-electron microscopy (cryo-EM) reconstructions with computational tools currently enables the interpretation at near-atomic resolution of different functional states of macromolecules, thereby deciphering the functional mechanism of biologically relevant complexes. Here, we present our integrative approaches to retrieve structural information recently extended as plugins in the standard molecular viewer Chimera. Examples of rigid-body or flexible fitting of the available atomic models into selected cryo-EM experimental maps will demonstrate the power of our methodology. This includes the structure of the NAIP5-NLRC4 inflammasome complex and its activation by the bacterial flagellin.

S5.3 Investigating the carbohydrate preferences of cellulolytic bacteria

ANA LUISA CARVALHO,^{1,§} Diana Ribeiro,¹ Aldino Viegas,¹ Benedita Pinheiro,¹ Virgínia Pires,² Filipa Marcelo,¹ Eurico Cabrita,¹ Maria João Romão,¹ Angelina Palma,¹ Carlos Fontes.²

¹*UCIBIO-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Caparica, Portugal;* ²*CIISA/FMV-UL, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal.*

[§]almc@fct.unl.pt

Some cellulolytic bacteria are remarkably efficient in the degradation of the intricate network of polysaccharides that constitute the plant cell wall. The driving force is the excretion and assembly of multi-enzyme complexes of catalytic and non-catalytic modules that work together as a megaDalton machinery: the Cellulosome. The enzymes' activity is potentiated by non-catalytic modules that specifically recognize the polysaccharides (the CBMs), while the whole assembly is maintained by the attachment of dockerin modules (part of the enzymes) to cohesin modules from a scaffolding protein. X-ray crystallography is the methodology that has been providing structural information about the interactions of these modules, either protein-protein^{1,2,3} or protein-carbohydrate⁴ and also about the catalytic sites of the enzymes⁵. In the absence of crystal structures, STD-NMR has proven useful in giving molecular details of specificity⁶. Furthermore, the structure of a partial cellulosomal assembly has also been achieved by cryo-EM⁷. More recently, crucial information that guides the production of complexes of interest can be given by carbohydrate microarray screening analysis⁸.

Financial support: UID/Multi/04378/2013, POCI-01-0145-FEDER-007728, PTDC/BBB-BEP/0869/2014, RECI/BBB-BEP/0124/2012.

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S5.4 Molecular architecture of the elongator catalytic sub-complex and its tRNA interaction

MARIA I. DAUDEN,^{1,§,§} Marcin Jaciuk,¹ Jan Kosinski,² Felix Weis,¹ Christoph W. Mueller,¹ Sebastian Glatt.³

¹European Molecular Biology Laboratory, Heidelberg, Germany; ²Centre for Structural Systems Biology, EMBL-Hamburg, Germany; ³Max Planck Research Group at the Malopolska Centre of Biotechnology, Jagiellonian University, Krakow, Poland.

§dauden@embl.de, §EBSA bursary

The highly conserved eukaryotic Elongator complex performs specific chemical modifications on wobble base uridines of tRNAs, which are essential for proteome stability and homeostasis (Nedialkova, 2015). Elongator is of fundamental clinical relevance, as mutations affecting its integrity and activity are related to the onset of neurodegenerative diseases, cancer and intellectual disabilities. The Elongator is formed by two copies of each of its six individual subunits (Elp1-6) that are all important for its tRNA modification activity (Huang, 2005). Although the overall architecture of the Elongator has been proposed using an integrative approach (Dauden, 2017), high resolution information of both the Elp123 catalytic sub-complex and the Elongator is still missing. Moreover, the detailed chemistry of the Elongator modification reaction is insufficiently described. Here we show the Elp123 structure at 3.3 Å resolution solved by cryo-electron microscopy, revealing novel structured parts that may be implicated in tRNA binding. We prove that Elp123 sub-complex is able to bind tRNA in the absence of the Elp456 sub-complex. Finally, we characterize this interaction in molecular detail solving the structure of the Elp123-tRNA complex also by cryo-EM. These structures provide the structural framework to understand the tRNA binding and modification in molecular detail.

S5.5 Theoretical description of ssDNA adsorption on carbon nanotubes

SHUSHANIK TONOYAN,[§] Davit Khechoyan, Vardges Mambreyan, Vladimir Morozov, Yevgeni Mamasakhlisov.

Yerevan State University, Yeveran, Armenia.

§sh.tonoyan@gmail.com

A series of experimental studies show, that single-stranded DNAs (ssDNA) form a stable complex with Single-walled carbon nanotubes (SWCNT) which uses successfully for dispersion and structure-based sorting of SWCNTs into an aqueous solution. Inspired by these experiments we investigated the ssDNA/SWCNT complex formation theoretically. It is shown, that thermodynamics and kinetics of CNT –DNA nanohybrid formation can be addressed in terms of models similar to those, describing the helix-coil transition in biopolymers. To address the assembly and disassembly of DNA/SWCNT we applied an approach developed earlier for the description of conformational transition in two-strand polynucleotides and polypeptides. The approach is formulated in terms of GMPC, which is the Potts-like model with multi-particle interaction. We adopted the analytical model to describe the adsorption of the ssDNA and dsDNA molecules on the surface of substantially one – dimensional SWCNT. The results were compared with experimental data. It is shown that the proposed theoretical model can be developed for the account of the effect of stacking-interaction, single-strand rigidity, energy of adsorption, sequence heterogeneity, etc. Theoretical results obtained for the homogeneous sequences are in good agreement with experiment.

S5.6 Characterizing protein (un)binding rates at the single-molecule level using lateral magnetic tweezers combined with tfrf microscopy

JULENE MADARIAGA-MARCOS,^{1,§,§} Cesar L. Pastrana,¹ Gemma L. M. Fisher,² Mark S. Dillingham,² Fernando Moreno-Herrero.¹

¹Department of Macromolecular Structures, National Center of Biotechnology, CSIC, Madrid, Spain; ²DNA:Protein Interactions Unit, School of Biochemistry, University of Bristol, Bristol, United Kingdom.

§jmadariaga@cnb.csic.es, §Best oral communication –“L. Camoens” prize, SBE bursary

Combining single-molecule techniques with fluorescence microscopy permits correlating mechanical measurements with directly visualized DNA:protein interactions. Specifically, the combination of magnetic tweezers (mt) with total internal reflection fluorescence (tfrf) microscopy is advantageous because one can follow many DNA molecules taking advantage of the high signal-to-noise ratio this fluorescence technique achieves. We have previously shown how to calibrate forces in this experimental configuration as well as its imaging capabilities to study parB, a protein involved in bacterial chromosome segregation¹. We have recently combined this hybrid setup with a double-channel laminar flow microfluidic device that is able to alternate between reagents (ie., buffer, protein, ATP) in a very precise way. This allows to study the binding/unbinding of proteins in a repetitive way at the single-molecule level, and we anticipate that it will allow to calculate binding and unbinding rates (k_{on} , k_{off}). Ongoing experiments include calculating binding and unbinding rates for ParB and the C-terminal domain of ParB (ParB^{Ctd}), which plays an important role in unspecific DNA-binding². Our results support previously proposed models on ParB and ParB^{Ctd}.

References

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S6 | Computational and Systems Biophysics

Hall Auditorium

Chair 1 ÓSCAR MILLET

Chair 2 CLAUDIO SOARES

Date: Friday, June 22nd

Time: 09:00–11:00

S6.1 Prediction of solution properties of globular and partially disordered proteins and nucleic acids

JOSE GARCÍA DE LA TORRE,[§] José Ginés Hernández-Cifre, Diego Amorós, Álvaro Ortega.
Universidad de Murcia, Murcia, Spain.

[§]jgt@um.es

Measurements of solution properties are useful to determine or validate bio-macromolecular structures. A theoretical/computational formalism is required to establish the structure-properties relationship. For folded quasi-rigid, globular proteins, we have developed a method, implemented in the computer program HYDROPRO [1], which allows the calculation of hydrodynamic and scattering properties from atomic- or residue- level structures, specified by atomic coordinates in a PDB. The accuracy of the prediction has been evaluated against experimental data, with a precision of the same order as experimental uncertainties [2]. The method can be also applied to rigid nucleic acids, particularly small oligonucleotides. The case of partially, intrinsically disordered structures is more complex; the formalism must include, in addition to calculation of properties, the generation of multiple conformations of the flexible structure. SIMUFLEX [3], a general program for simulation of conformation and properties, has been adapted as to include a coarse-grained model and force field for proteins [4]. Additional, ancillary bioinformatics tools are employed to construct the input file for SIMUFLEX. This complex but well-structured scheme yields excellent agreement with experiments.

Financial support: Fundación Séneca 19353/PI/14; MINECO CTQ2017-85425-P

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S6.2 Prediction and inference in complex biophysical systems

JOSÉ M. VILAR.[§]

UPV/EHU, Bilbao, Spain.

[§]j.vilar@ikerbasque.org

The traditional computational systems approach seeks to provide the emergent collective behavior from the properties of the constituent elements. The molecular and mechanistic complexity of typical biophysical systems, however, often results in a nonexistent, deficient, or misleading characterization of the underlying molecular components and their interactions. Here, I will discuss the state of the art of novel approaches that function in reverse, namely, that use the emergent behavior to infer precise properties of the molecular components and that are able to make accurate predictions about the system behavior even without a basic molecular description. I will illustrate the main points with key examples in gene regulation, protein aggregation, and odor perception.

S6.3 Identifying binding sites on the mitochondrial VDAC protein by coarse-grain molecular dynamics

MANUEL N. MELO,^{1,2,§} Guilherme Razzera,^{1,4} Joost Holthuis,³ Siewert J. Marrink.²

¹*Instituto de Tecnologia Química e Biológica António Xavier - Universidade NOVA de Lisboa, Portugal;* ²*Groningen Biomolecular and Biotechnology Institute - University of Groningen, Groningen, The Netherlands;* ³*Molecular Cell Biology Division, University of Osnabrück, Austria;* ⁴*Departamento de Bioquímica, Universidade Federal de Santa Catarina, Brazil.*

[§]m.n.melo@itqb.unl.pt

The Martini coarse-grain molecular dynamics forcefield is a powerful tool to characterize protein-lipid and protein-protein interactions. I will showcase how Martini was successfully used to identify a lipid binding site on the mitochondrial voltage-dependent anionic channel (VDAC). The very long timescales afforded by the use of Martini further allowed the collection of thousands of binding/unbinding events, enough to quantitatively distinguish the binding behavior between two VDAC isoforms. Finally, I will describe a characterization of VDAC oligomerization: dimerization energy landscapes, largely compatible with experimentally-derived models, were obtained by the coupling of Martini to enhanced sampling methods. This approach enriches the picture of VDAC interactions in the mitochondrial membrane and allows the assessment lipid binding impact on VDAC oligomerization propensity.

S6.4 Qm/mm theoretical studies of enzyme catalyzed reaction

VICENT MOLINER,[§] Sergio Martí, Raquel Castillo, Katarzyna Swiderek, Maite Roca, Arafet Kemel, Manuel Delgado, Daria de Raffe, Natalia Serrano.

Universitat Jaume I, Castellón, Spain.

[§]moliner@uji.es

Computational chemistry techniques based on the combination of quantum chemistry and classical molecular mechanics (qm/mm) have been extensively applied to the study of enzyme catalysis. Merging these techniques with experimental methods has allowed to acquire a deep knowledge of the reaction mechanisms of these complex but highly efficient biocatalysts at the molecular level. We will focus in this communication on aspects such as the controversial debate on whether protein dynamics are linked to the chemical reaction step,[1] the role of the quantum tunnelling and the electrostatic effects contributions to catalysis,[2,3] the understanding of enzyme promiscuity and its applications[4] or the relevance of compression effects in enzymatic methyl transfer reactions [5].

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S6.5 Quantitative assessment of methods often used to obtain rate constants from biomolecular simulations – translocation of cholesterol across a lipid bilayer

HUGO A.L. FILIPE,^{1,2,§,§} Matti Javanainen,³ Armindo Salvador,² Adelino Galvão,⁴ Ilpo Vattulainen,^{3,5} Luís M. S. Loura,^{1,6} Maria João Moreno.^{1,7}

¹Coimbra Chemistry Center, University of Coimbra, Portugal; ²Center for Neuroscience and Cell Biology, University of Coimbra, Portugal; ³Laboratory of Physics, Tampere University of Technology and Department of Physics, University of Helsinki, Finland; ⁴Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Portugal; ⁵MEMPHYS – Center for Biomembrane Physics, Denmark; ⁶Faculty of Pharmacy, University of Coimbra, Portugal; ⁷Chemistry Department, University of Coimbra, Portugal.

[§]hugolouofilipe@gmail.com, [§]EBSA bursary

Calculation of rate constants of chemical processes from molecular dynamics (MD) simulations has been a long-sought but elusive goal. This problem is particularly relevant in processes occurring in biological systems, such as the translocation across biomembranes, the rate limiting step in the permeation of most drugs. While several formalisms have been proposed to calculate these rate constants, their applicability has not been critically evaluated. This work presents this assessment. To this end, we first used unbiased coarse-grained MD simulations to generate a large set of spontaneous events for the translocation of cholesterol across 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine bilayers, allowing us to determine the reaction coordinate, the rate, and the molecular mechanism of cholesterol's translocation in great detail. In this context, a novel procedure was also employed to obtain an effective rate constant, based on transitions between different states along the reaction coordinate. These quantitative data were then compared with the predictions of several available formalisms to assess their accuracy. While most of the tested formalisms lead to results in reasonable agreement with the effective rate constant, one of the methods was superior. This technique is based on explicit relaxation frequencies from the transition state in the forward- and backward-directions along the reaction coordinate.

S6.6 The development of new diagnostics for skin cancer

CHRISTINA KAMMA-LORGER, Adriá Berrocal, Eduardo Solano-Minuesa, Sílvia Gil, Manel Sabés.

BL11-SWEET, ALBA Synchrotron Light Source, Barcelona, Spain.

Skin cancer is the most common type of cancer and sums up more than all other cancers combined. There is a worrying increase of skin cancer incidents with melanoma being the most serious one. In the United States of America (USA) the number of diagnosed cases of melanoma has doubled between 1982 and 2011 and it is predicted that in 2017 10.000 people are in risk of dying. The risk of metastasis, poses life threatening risks to affected individuals. Early detection and complete excision of the cancerous tissue is key in preventing cancer from spreading. Skin pathology, like cancer, is always accompanied with a compromise on collagen integrity in the tissue. The objective of this project is to use X-Rays and in particular small angle scattering (SAXS) methods in order to detect changes in collagen structure that will imply the spread of the disease in the skin. This approach will be used for the design of diagnostic tools that will provide clinicians with a precise map of the skin tumor indicating its extent and hence the amount that has to be removed in order to eliminate the disease completely from affected parts of the skin.

S6.7 Computational study of the retro-aldol de novo designed enzymes

SERGIO MARTÍ-FORÉS,[§] Daria de Raffe, Vicent Moliner.

Universitat Jaume I, Castellón, Spain.

[§]smart@uji.es

Nowadays there are two major complementary strands for the development of new enzyme-based catalysts: directed evolution and rational design. The first one is based on performing random mutations and recombinations, followed a screening process. One of the advantages of this technique is that prior structural knowledge is not necessary, but a threshold of activity with respect to the reaction that is intended to catalyze to initiate the process is essential. On the other hand, rational design allows to introduce directed mutations and analyze their impact on the overall process, although it is crucial to have the structural information of the protein (X-ray structure). Over the time, three strategies or starting points have been adopted for conducting the rational design: i) use of immuno-globulins (catalytic antibodies), ii) promotion of the enzymatic promiscuity (boosting of secondary reactions) and iii) de novo design (or theozymes). In the present work, we have carried out a computational study of a set of theozymes designed to catalyze the retro-aldolic reaction. This reaction consists of a multi-stage process leading to a C-C bond breaking, for which we intend to shed light on the reaction mechanism and improve the catalytic efficiency of the process.

Aw | 2018 SBE and SPBf Prizes

Hall Auditorium

Chair 1 JESÚS PÉREZ-GIL

Chair 2 NUNO C. SANTOS

Date: Friday, June 22nd

Time: 15:00-17:15

Manuel Rico – Bruker Prize

Aw.1 Exploring the molecular determinants of ligand recognition and binding: from chemical structure to mechanisms of drug action

F. JAVIER LUQUE. §

University of Barcelona, Barcelona, Spain.

§ fjluque@ub.edu

Dr. F. Javier Luque is Full Professor in Physical Chemistry at the University of Barcelona. He obtained his PhD degree in Chemistry at the University Autònoma de Barcelona in 1989. He spent research periods at the Swiss Federal Institute of Technology, University of Pisa and University of Nancy. In 2002 he was awarded the Catalan Distinction for the Promotion of University Research for Young Scientists, and the ICREA Academia award in 2012. He is leading the Computational Biology and Drug Design group in the Institute of Biomedicine, and is also member of the Institute of Theoretical and Computational Chemistry at the University of Barcelona. The main focus of his research is the study of biomolecular systems using the theoretical and computational methods, with a special emphasis in drug discovery.



Drug design relies on the concept that a small molecule interacts with a physiological target in such a way that the strength of the interaction determines the biological response. This assumption relies at the heart of structure-based drug design strategies. Despite the efforts in developing novel structure-based approaches and the integration within multidisciplinary research that combines molecular biology, biophysics, high-throughput screening and genomics, it is paradoxical that the number of chemical entities released in the last years has risen only slightly (Nat. Rev. Drug Discov. 2011, 10, 507). This reflects our still limited understanding of the molecular determinants and mechanisms of drug activity and the complexity of biological systems (Nat. Rev. Drug. Discov. 2015, 14, 95). In this context, this talk will highlight several challenging aspects of the molecular modeling of ligand-target interactions. Specifically, attention will be paid to the relationship between structural and physicochemical features of small molecules, the mechanism that underlies their recognition and binding to macromolecular targets, and the modulation of the biological response.

E. Pérez-Payá – SBE40 Prize

Aw.2 Sensing matrix rigidity: transducing mechanical signals from integrins to the nucleus

PERE ROCA-CUSACHS. §

Institute for Bioengineering of Catalonia, Barcelona, Spain.

§ proca@ibecbarcelona.eu

Dr. Pere Roca-Cusachs obtained his PhD in cellular biophysics in 2007 from the Medical School at the University of Barcelona. He then worked in the lab of Prof. Michael Sheetz (Department of Biological Sciences, Columbia University) as a post-doctoral researcher until 2011. In 2011, he joined the University of Barcelona, where he is now an associate professor. In 2012, he obtained a joint position as group leader at the Institute for bioengineering of Catalonia (IBEC). The research of his group focuses on unraveling the physical and molecular mechanisms by which cells detect and respond to mechanical signals. His contributions include the discovery of mechanisms explaining transcription factor mechanosensitivity (Elosegui-Artola et al., Cell 2017) or cell sensing of extracellular matrix properties such as mechanical rigidity (Elosegui-Artola et al., Nat. Cell Biol. 2016) and ligand distribution (Oria et al., Nature 2017). He is currently the coordinator of an EU-funded Future and Emerging Technologies (FET) project dedicated to understanding the mechanical control of biological function. He is a recipient of the EMBO Young Investigator award, and the 2017 City of Barcelona award to the life sciences.



Cell proliferation and differentiation, as well as key processes in development, tumorigenesis, and wound healing, are strongly determined by the rigidity of the extracellular matrix (ECM). In this talk, I will explain how we combine molecular biology, biophysical measurements, and theoretical modelling to understand the mechanisms by which cells sense and respond to matrix rigidity. I will discuss how the properties under force of integrin-ECM bonds, and of the adaptor protein talin, drive and regulate rigidity sensing. I will further discuss how this sensing can be understood through a computational molecular clutch model, which can quantitatively predict the role of integrins, talin, myosin, and ECM receptors, and their effect on cell response. Finally, I will analyze how signals triggered by rigidity at cell-ECM adhesions are transmitted to the nucleus, leading to the activation of the transcriptional regulator YAP.

SPBf Young Investigator Award

Aw.3 The direct role of selenocysteine in (NiFeSe) hydrogenase maturation and catalysis

MARTA COIMBRA-MARQUES,^{1,§} Cristina Tapia,² Óscar Gutiérrez-Sanz,³ Ana Raquel Ramos,³ Kimberly L. Keller,¹ Judy Wall,⁴ Antonio L. de Lacey,⁴ Pedro M. Matias,³ Inês A. C. Pereira.¹

¹Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Oeiras, Portugal;

²Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal; ³Instituto de Catálisis y Petroleoquímica, CSIC, Madrid, Spain; ⁴Biochemistry Department, University of Missouri, Columbia, MO, USA and ENIGMA (Ecosystems and Networks Integrated with Genes and Molecular Assemblies), Berkeley, CA, USA.

§Martamarques@medicina.ulisboa.pt

Dr. Marata Marques graduated in Biological Engineering (2007) from *Instituto Superior Técnico, Universidade Nova de Lisboa* (Portugal). In 2008 she started as a research student at the ITQB NOVA, under the joint supervision of Dr. Pedro Matias and Dr. Inês Cardoso-Pereira. In 2010 she obtained a Ph.D. fellowship from the FCT, with a project dedicated to the biochemical and structural characterization of a highly active hydrogen-producing enzyme, using biochemistry, biophysics, spectroscopy (UV, UV-Vis and IR), structural biology (X-ray) and molecular biology techniques. She completed her Ph.D. in 2015 with important achievements about the understanding and manipulation of enzymatic production of H₂, including the successful in vitro integration of nickel (catalyst) into the native active site of the protein, as well as the role of selenium in maturation and catalytic properties of the hydrogenase enzyme. This research resulted in 19 publications in international, high-impact journals in the last 8 years. She is currently working as a Postdoctoral Research Fellow at the *Instituto de Medicina Molecular – iMM João Lobo Antunes (Universidade Nova de Lisboa)*.



Hydrogenases are highly active enzymes for hydrogen production and oxidation. [NiFeSe] hydrogenases, where selenocysteine is a ligand to the active site Ni, have high catalytic activities and a bias for H₂ production. In contrast to [NiFe] hydrogenases, they display reduced H₂ inhibition and are rapidly reactivated after contact with oxygen. Here, we report a homologous expression system for production of recombinant [NiFeSe] hydrogenase from *Desulfovibrio vulgaris* Hildenborough, and study of a Sec489Cys variant where for the first time a [NiFeSe] hydrogenase was converted to a [NiFe] type. This modification led to a severely reduced Ni incorporation, revealing the direct involvement of this residue in the maturation process. The Ni-depleted protein could be partly reconstituted to generate an enzyme displaying much reduced activity and inactive states characteristic of [NiFe] hydrogenases. The Ni-U489C variant shows that selenium plays a crucial role in protection against oxidative damage and the high catalytic activities of the [NiFeSe] hydrogenases.

Antalgenics – SBE33 Prize

Aw.4 From single molecule to single cell: the power of one

JOAN CAMUÑAS.[§]

Stanford University, Stanford, CA, USA.

[§] jcamunas@stanford.edu

Dr. Camuñas-Soler obtained his B.Sc. in Physics in the University of Barcelona and M. Sc. in Biophysics in the Royal Institute of Technology (KTH) in Stockholm. In 2015, he obtained his PhD under the supervision of Prof. Felix Ritort in the University of Barcelona, where he combined single-molecule methods and non-equilibrium physics to study binding reactions between nucleic acids and small ligands. In this work, he contributed to the formulation and experimental validation of non-equilibrium laws governing the dynamics of biomolecular systems. He also developed new experimental methods using optical tweezers to measure the selectivity, thermodynamics and kinetics of small anticancer agents that target DNA. His doctoral work has been recognized through several awards such as the 'XXI Premi Claustre de Doctors' from University of Barcelona. As a postdoctoral scholar in Stephen Quake lab (Stanford University), he is extending his field of expertise to the physics of larger biological systems, such as the cell and the genome. He is currently developing novel tools to dissect the relationship between the biophysical properties of cells and their genetic content. In particular he is combining electrophysiological measurements (patch-clamp) with single-cell RNA sequencing, to link the transcriptome of a cell to its functional response as observed from its electrical activity. He is currently using this set-up to characterize the functional response of human pancreatic cells and determine the genetic signatures explaining cellular dysfunction in diabetes. He combines this work with projects aiming to detect nucleic acids in blood as a tool for non-invasive diagnostics.



Novel technologies enable us to probe biological systems at high resolution and follow individual molecular or cellular reactions. In the first part of my talk, I will discuss force spectroscopy methods for tracking binding reactions between small ligands and DNA at the single-molecule level. I will focus on the use of optical tweezers to mechanically disrupt ligand-DNA complexes and discuss the use of statistical physics tools to measure the selectivity, thermodynamics and kinetics of molecular interactions. In the second part of my talk, I will introduce ongoing work that combines single-cell biophysics and genomics. I will show how the combination of electrophysiological measurements (patch-clamp) and single-cell RNA sequencing can be used to assess the functionality of individual human pancreatic cells. These unprecedented experiments link the transcriptome of a cell to its function (ion channel activity, exocytosis), thus providing access to signatures of cellular dysfunction in diabetes. Taken together, my work on single-molecule and single-cell approaches enables us to interrogate biological heterogeneity in unprecedented ways.



Posters

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PO | Physics in Biology

Hall Posters and Exhibitors Hall

Odd-numbered posters

Date: Thursday, June 21st

Time: 19:00-20:00

Even-numbered posters

Date: Friday, June 22nd

Time: 12:00-13:00

PO.1 Hydrophobicity of halogenated benzotriazole derivatives. Application of density measurements to determine the free energy of hydration.

ANNA MARIA SZYMANIEC,[§] Ewa Bugajska, Kinga Mieczkowska, Sławomir Kasperowicz, Jarosław Poznanski.

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Department of Biophysics, Warsaw, Poland.

[§]szymaniec@ibb.waw.pl

Hydrophobic effect is the observed tendency of nonpolar substances to organize surrounding water molecules maximizing hydrogen bonding between them. This effect occurs to be extremely important in terms of drug design, especially for highly hydrophobic ligands, binding ability of which strongly depends on their adverse energetic interactions with aqueous solvent. Inhibitory activity of halogenated compounds can be measured by overall free energy of protein-ligand binding process. It can be divided into electrostatic interactions, including halogen bonds, and hydrophobic interactions. To calculate the overall free energy, all the contributions must be determined. We propose that free energy of hydration can be estimated directly from the disagreement between molecular volumes calculated theoretically with that estimated experimentally. The obtained thermodynamic parameters can be further used for better characterization of potential drug candidates. This work was supported by NCN grant 2015/19/B/ST4/02156 and School of Molecular Biology IBB PAN. The equipment was sponsored in part by Centre for Preclinical Research and Technology (CePT).

P0.2 Distinct mechanisms for blocking the activity of pestivirus viroporin P7 revealed by ion-channel activity and single vesicle permeability

ANTONIO ALCARAZ,^{1,§} Eneko Largo,² Douglas P. Glaude,³ Johana Torralba,² Vicente M. Aguilera,¹ Manuel V. Borca,³ José L. Nieva.²

¹Laboratory of Molecular Biophysics. Department of Physics, University Jaume I, Castellón, Spain; ²Biofisika Institute (CSIC, UPV/EHU) and Biochemistry and Molecular Biology Department, University of the Basque Country (UPV/EHU), Spain; ³Plum Island Animal Disease Center, ARS, USDA, Greenport, USA.

§alcaraza@uji.es

Permeabilization of the endomembrane system by viroporins is instrumental in the progression of host-cell infection by many viral pathogens. Thus, blocking/attenuation of viroporin activity provides a generic methodology for antiviral and vaccine development. The permeabilization of membranes emulating the Endoplasmic Reticulum by the CSFV viroporin p7 depends on two sequence determinants: the pore-forming C-terminal, and the preceding polar loop that regulates its activity. Here, by combining ion-channel electrophysiology with imaging of single Giant Unilamellar Vesicles (GUVs), we demonstrate that point substitutions directed to conserved residues within these regions hamper virus production in cells following distinct mechanisms. Whereas the polar loop appeared to be involved in protein insertion and oligomerization, substitution of residues predicted to face the lumen of the pore inhibited large conducting channels over smaller ones. Quantitative analyses of the ER-GUV distribution as a function of the solute size revealed a selective inhibition for the permeation of solutes demonstrating that the mutation targeting the pore-domain actually altered the dimension of the p7 pores. Collectively, our data suggest that p7 viroporin may assemble into nanometric pores. Moreover, the observation that specific mutations can interfere with formation of the larger pores suggests prospective strategies to block/attenuate pestiviruses by targeting viroporin p7.

P0.3 Quantification of protein copy number from super-resolution images

CARLO MANZO,^{1,§} Francesca Cella-Zanacchi,² Nathan D. Derr,³ Maria F. García-Parajo,⁴ Melike Lakadamyali.⁵

¹Universitat de Vic - Universitat Central de Catalunya, Vic, Spain; ²Instituto Italiano di Tecnologia, Genova, Italy; ³Smith College, Northampton, Massachusetts, USA; ⁴ICFO - Castelldefels (Barcelona), Spain; ⁵University of Pennsylvania, Philadelphia, Pennsylvania, USA.

§carlo.manzo@uvic.cat

Single-molecule-based super-resolution microscopy offers researchers a unique tool to visualize biological processes at the nanoscale. Nevertheless, providing a quantitative description of the molecular mechanisms underlying cellular function requires the precise molecular counting of protein copy numbers. Suitable calibration methods - based on the combination of biochemicals and analytical tools - represent a valuable solution to address the challenges of molecular counting using several super-resolution techniques (STORM, STED) in conjunction with immunofluorescence. Along this line, we have recently proposed a versatile platform for calibrating fluorophore and antibody labeling efficiency based on DNA origami and GFP antibodies to quantify protein copy number in cellular contexts using localization microscopy. The combination of this calibration with image and data analysis methods, besides quantifying the average protein copy number in a cell, allows determining the abundance of various oligomeric states [1]. These quantitative approaches allow accurate studies of the stoichiometry of membrane proteins, nucleoporins and molecular motors.

References

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P0.4 Modular proteins as scaffolds for self-assembled hybrid nanostructures.

ELENA LÓPEZ-MARTÍNEZ,^{1,§,§} Antonio Aires,¹ Sara H. Mejías,² Pierre Couleaud,² Ana Martín-Lasanta,³ Cristiana Extremiana,² David Romera,² Manuel Rodríguez,² Teresa González,² Aitziber L. Cortajarena.¹

¹*CIC biomaGUNE, San Sebastián, Spain;* ²*Imdea Nanociencia, Madrid, Spain;* ³*Departamento de Química Orgánica, Universidad de Granada, Granada, Spain.*

§elopez@cicbiomagune.es, §*SBE bursary*

Protein engineering in combination with nanotechnology stands as a powerful tool for developing biomaterials with unique properties. These protein-based materials can be designed using a rational approach for optimizing the desired properties. The use of bottom-up construction and self-assembly principles allows an easy control of the organization of different functional elements on protein structures. Repeat proteins are optimal systems for this endeavour due to their modular organization and their potential functional diversity. Our approach consists on using consensus Tetratricopeptide repeat (CTPR) proteins, based on a 34 amino acids motif, because they can be easily combined using their modularity to build designed structures. This work develops several strategies to use CTPR proteins as scaffolds for the conjugation of different molecules in order to explore novel functionalities. On one hand we use gold nanoparticles (AuNPs) conjugated to CTPR proteins for organizing conductive structures at nanometric scale with uses in nanoelectronics and plasmonics. On the other hand, we conjugate CTPR proteins with photoisomerizable molecules for the photocontrol of the structure of the protein, with the aim of building a general toolkit for protein switches which functionalities can be turned on and off using light.

P0.5 Halogen bonding in protein-ligand systems. Application of site-directed mutagenesis of the ATP binding site of the catalytic subunit of the protein kinase CK2.

EWA BUGAJSKA.[§]

Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Department of Biophysics, Warsaw, Poland.

§ewa.b@ibb.waw.pl

CK2 is constitutively active Ser/Thr protein kinase. We use the catalytic subunit of human CK2 (hCK2a) as the model protein to be targeted by low-mass ligands. Human CK2a carries a peculiar stretch of histidine residues, most of which are highly conserved in the CMGC group of protein kinases. Our previous studies showed that His160, which is proximal to the ATP binding site, affects pH-dependent affinity for ligand binding. Site-directed mutagenesis was applied to replace His160 by either Gln or Phe. Interaction of the wild type hCK2a, and its two variants, with the series of halogenated benzotriazoles was analyzed by means of thermal shift assay approach. In the case of small rigid solutes, a ligand-induced change in the temperature of protein unfolding evidences the binding phenomena. His160Gln replacement significantly increases protein thermal stability, but also affects preferences towards particular ligands. The proposed approach based on combination of molecular biology and optical spectroscopy methods enables decoupling of electrostatic and halogen bonding contributions to the intermolecular interactions in protein-ligand systems.

Financial support: This work was supported by NCN grant 2017/25/B/ST4/01613. The equipment used was sponsored in part by the Centre for Preclinical Research and Technology (CePT).

P0.6 Single-molecule translocations process through glass nanocapillaries

M. Rico-Pastó,¹ C. Verriere,¹ I. Pastor-del-Campo,² M. Manosas,¹ F. RITORT.^{1,2,§}

¹Facultat de Física, Universitat de Barcelona, Barcelona, Spain; ²CIBER-BNN, Madrid Spain.

§fritort@gmail.com

Glass nanopore technology employs a nanoscale hole on the tip of a glass tub to stochastically sense with high throughput individual biomolecules in solution. Recent studies have been used glass nanocapillaries to discriminate different species of a protein [1]. In single-molecule translocation experiments with glass nanocapillaries (tip holes in the range 10-100 nm), changes in ionic current are used to recognize molecular motifs from current blockade events [2]. In this work we have characterized the translocation process of a linear double-stranded DNA molecule (48.5kbp) through pores of sizes 50-80nm. We observe that the random coil of gyration radius ~630nm enters the nanohole by pulling the DNA from one of the two extremities. By hypothesizing that the magnitude of the current blockade is proportional to the cross-section of the DNA double helix we find a diameter equal to 2.1 ± 0.6 nm. Moreover, we have observed that the mean translocation time, which is the residence time of the blockade current event, is independent of the pore size. The translocation rate is found to follow an Arrhenius-type activated behaviour as a function of the applied voltage. We have determined the attempt translocation rate, 19 ± 1 s⁻¹, and the distance to the transition state, 0.15 ± 0.02 e⁻.

References

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2. Kaikai Chen, Matyas Juhasz, *et al. Nano Lett.* (2017) 17 (9).

P0.7 A home-made simultaneous AFM+TIRFM microscope for single molecule physical virology

FRANCISCO JOSÉ MORENO-MADRID,^{1,§} Pablo Ares,¹ Ignacio Horcas,¹ Iwan Schaap,² Trevor Douglas,³ P. J. de Pablo,¹ J. Gómez-Herrero.¹

¹Departamento de Física de la Materia Condensada. Universidad Autónoma de Madrid, Madrid, Spain; ²Heriot-Watt University, Edinburgh, U.K; ³Chemistry Department, Indiana University, Bloomington, U.S.A..

§paco.jalcama@gmail.com

Atomic Force Microscopy (AFM) is a powerful tool to unravel several physical properties of virus-like particles (VLPs) at the single molecule level in liquids [1]. However, a sine qua non requirement is the adsorption of the specimen on a surface that hampers the investigation of molecular phenomena occurring off this surface. In the case of virus shells, those include either natural (genome) or artificial cargo diffusion after leaving the protein container. These new problems require the implementation of additional microscopy techniques in the system. Total Internal Reflection Fluorescence Microscopy (TIRFM) is a perfect tool to combine with AFM [2]. Here, a home-made simultaneous AFM+TIRFM microscope is presented, in which imaging soft biological samples in liquid implies some challenges in the design of the AFM part [3][4]. This has made it quite different from other AFM systems in the lab so far. In order to show our system proof of concept, we present high resolution AFM images of P22 bacteriophage and a fluorescent study of eGFP proteins packed inside.

References

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P0.8 Nodularin cyanotoxin molecular characterization and detection using surface enhanced raman scattering (SERS) and drop coating deposition raman (DCDR)

IOANA BREZESTEAN,[§] David Leontin, Vasile Chis, Reka A. Domokos, Simona Cinta Pinzaru.
Babes Bolyai University, Cluj-Napoca, Romania.

[§]ioana_brezestean@yahoo.com

Nodularin is potent toxin produced by cyanobacterium *Nodularia Spumigena* [1] and its occurrence along with other microcystins, a class of cyanotoxins secreted by certain cyanobacteria pose difficulty for identification when mixed toxins co-exist in environmental waters. A previous analytical Raman study [2] demonstrated the possibility of their differentiation based on Raman peaks ratio and principal component analysis (PCA) when pure toxins were considered. We report here the Raman spectra of nodularin using drop coating deposition Raman (DCDR) micro-spectroscopy and surface-enhanced Raman scattering (SERS). An ampule of 100 mg nodularin was dissolved in ethanol as starting solution and several diluted concentrations have been prepared, from 1.212×10^{-3} to 1.121×10^{-7} mol/L. The DCDR process of 5-25 μ L that generated solid sample recrystallized micro-deposits on a hydrophobic plate further used for micro-Raman spectroscopy. SERS solutions were prepared using 10 μ l of each nodularin solutions added to 200 ml colloidal Ag nanoparticles prepared by citrate-reduced method. SERS spectra showed prominent bands at 950, 1027, 1379, 1566 cm^{-1} . We used the relative SERS intensity ratios $R_1 = I_{1566}/I_{1633}$ and $R_2 = I_{223}/I_{883}$ to assess the concentration dependence SERS signal. The plotted $R_{1,2}$ values against concentration allowed obtaining calibration SERS curve for quantitative determination.

P0.9 Study of the interaction of small drugs with DNA using single-molecule footprinting

ISABEL PASTOR-DEL-CAMPO,^{1,2,§} Marc Rico-Pastó,¹ Fèlix Ritort.^{1,2}

¹*Small Biosystems Lab, Universitat de Barcelona, Barcelona, Spain;* ²*CIBER-BBN, Instituto de Salud Carlos III, Madrid, Spain.*

[§]i.pastordelcampo@gmail.com

In this work, we have studied the interaction of Netropsin with DNA hairpins and dsDNA using unzipping and stretching single-molecule experiments. Netropsin is a small drug which binds to the DNA minor groove. It is reported that binds in AATT DNA motives specifically. However, our unzipping results suggest that Netropsin not only binds on a specific motive, but it binds along the entire minor groove of DNA molecule, and its interaction is stronger in AT rich regions. This is proved in the observation of an increasement of the mean unzipping force in presence of Netropsin. Moreover, in this work we have proved that Netropsin does not bind with ssDNA. Finally, in these experiments we have observed that the refolding pattern is modified just when the hairpin starts to be folded in presence of Netropsin. This result suggest that Netropsin binds to DNA minor groove during the hairpin formation. On the other hand, from stretching experiments the interaction of Netropsin with dsDNA yields a rise in the persistence length but not in the interphosphate distances, which can be ascribed to an overtwist phenomenon in the dsDNA due to the presence of this drug as it is suggested in the literature.

P0.10 Reconstruction of ancestral enzymes for lignocellulosic biomass degradation

LEYRE BARANDIARÁN-LARREA,[§] Borja Alonso Lerma, Nerea Barruetabeña Garate, Raúl Pérez-Jiménez.

Nanobiomechanics group, CIC nanoGUNE, San Sebastián, Spain.

[§]lbarandiaran@nanogune.eu

The hydrolysis of lignocellulosic biomass offers valuable products such as nanocellulose, lignin and biofuels. The breakdown of biomass can be achieved using a combination of enzymes such as cellulases, laccases, xylanases or lytic polysaccharide monooxygenases (LPMO). Nevertheless, performing enzymatic reactions in industrial conditions is still a challenge. In our lab, we use Ancestral Sequence Reconstruction (ASR) as an effective method to improve some features of lignocellulosic enzymes for their biotechnological applications. ASR allows us to track the evolutionary history of genes and proteins to obtain information about extinct species that lived in the harsh environments of ancient Earth. Here, we have reconstructed a cocktail of bacterial enzymes that show outstanding temperature resistance and a wide pH range as well as a perfect chemical synergy to work together on different substrates. In addition, some of these enzymes activities have been further improved by generating structures called nanoflowers that incorporate the immobilized enzymes. Our results show that combining ASR with nanostructures for enzyme immobilization is a promising method for the catalytic breakdown of biomass.

P0.11 Access resistance dominates ion permeation through membrane channels in solutions of low conductivity

MARÍA LIDÓN LÓPEZ-PERIS,^{1,§} María Queral-Martín,² Vicente M. Aguilera,¹ Antonio Alcaraz.¹

¹*Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Castellón, Spain;* ²*Section on Molecular Transport, Eunice Kennedy Shriver NICHD, National Institutes of Health, Bethesda, MD, USA.*

[§]lopezp@uji.es

Ion transport at the nanoscale occurs under confinement conditions so that interfacial effects such as access resistance (AR) become relevant. Here we show that interpreting electrophysiological measurements in terms of channel ion selective properties requires the consideration of interfacial effects, up to the point that they dominate protein channel conductance in diluted solutions. We measure AR in a large ion channel, the bacterial porin OmpF, by means of single-channel conductance measurements in salt solutions with varying concentrations of high molecular weight PEG, sterically excluded from the pore. Comparison of experiments done in charged and neutral membranes shows that lipid surface charges modify the ion distribution and determine the value of AR, indicating that lipid molecules are more than passive scaffolds even in the case of large transmembrane proteins. We also found that AR might reach up to 80% of the total channel conductance in diluted solutions, where current recordings reflect essentially the AR of the system and depend marginally on the pore ion concentrations and selective properties. These findings have implications for several low aspect ratio biological channels that perform their physiological function in a low ionic strength and macromolecule crowded environment, just the two conditions enhancing the AR contribution.

PO.12 Influence of the osmolarity of the aqueous solution of irrigation on the growth of the sunflower plant

MAAMAR BOUKABCHA,^{1,§} Abd El Farid Djemai,² Abd El Kader Alibenamara.³

¹Research Laboratory in Biophysics and Medical Technology, Higher Institute of Medical Technology, Tunis, Tunisia;

²Faculty of physics, Mohamed Boudiaf University (USTO) of Oran, Algeria.; ³Faculty of exact sciences and computer, university of Chlef, Algeria..

§boukamaa@gmail.com

In this work, we studied the influence of the osmolarity of the aqueous solution which has utility for irrigation on the growth of sunflower plants in vivo and at the greenhouse of the variety: *Helianthus annuus*. Impacts of the different osmolarities that are used in milliosmol (m osmol) per litre and according to different sample are: 8.96; 9.82; 10.24; 10.67 and 12.38 on the in vivo growth of the sunflower plant. The test was installed in a greenhouse at a different temperature depending on the environmental climate in the greenhouse. Seeds were seeded into Goblet-shaped tubes of a cylindrical type in a soil medium of a type characteristic of the potting soil. Stem height, stem diameter and aerial biomasses, total were studied. The results obtained show that osmole stress exerted only statistically significant effects on epicotyl height, epicotyl stem diameter, and aerial biomass, indicating that *H. annuus* is a rare species. More tolerant to osmolarity, in the first stage after germination than at the plant stage.

PO.13 Heat capacity change across the DNA hybridization transition measured in single-molecule experiments

MARC RICO-PASTÓ,^{1,§} Marco Ribezzi-Crivellari,² Félix Ritort.^{1,3}

¹Departament de Física de la Materia Condensada, Universitat de Barcelona, Barcelona, Spain; ²École Supérieure de Physique et de Chimie Industrielles de la Ville de Paris, Paris, France; ³Ciber-BBN, Instituto de Salud Carlos III, Madrid, Spain; Instituto de Salud Carlos III, Madrid, Spain.

§m.rico.pasto@gmail.com

An accurate knowledge of the thermodynamic properties of nucleic acids as a function of temperature is crucial to predict their structure and stability far away from the physiological temperature. Traditionally, molecular thermodynamic properties, such as free energy, enthalpy and entropy change have been determined by bulk experiments. Nowadays, single molecule experiments have become powerful, accurate and bulk complementary methods to characterize thermodynamic parameters such as base pair (bp) energy contributions and folding free energies. We have determined the enthalpy and entropy change per bp as a function of temperature from hopping experiments. We have carried out experiment with three different DNA hairpins, poly-GC, poly-AT and CD4 (48% GC content), in a temperature range between 5 and 50°C. A strong temperature dependency have been observed for all the molecules, what it means a non-zero heat capacity change, ΔC_p . Our results highlight that the capacity change comes from the ssDNA. This was suggested from bulk experiments without a strong experimental evidence. Finally, we have compared the measured folding free energy at each temperature as $\Delta G = \Delta H - T\Delta S$ with the measured one by subtracting the stretching and orienting contributions using the WLC and FJC models.

P0.14 Scaling behavior for ion transport through protein and proteolipidic channels

VICENTE M. AGUILELLA,^{1,§} María Lidón López-Peris,¹ Noel Fructuoso,¹ María Queralt-Martín,² Antonio Alcaraz.¹

¹Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Castellón, Spain; ²Section on Molecular Transport, Eunice Kennedy Shriver NICHD, National Institutes of Health, Bethesda, MD, USA.

§aguilell@uji.es

The ionic conductance in membrane channels exhibits a power law behavior with respect to electrolyte concentration. By analyzing experiments performed in several protein channels we show that scaling behavior depends on several interconnected effects, being the distinction between them so subtle that the classical biphasic bulk+surface model fails. In fact, the presence of interfacial effects could give rise to an apparent universal scaling that hides the channel distinctive features. In the case of biological pores, we show also that the presence of interfacial effects could give rise to an apparent universal scaling that does not reflect the channel actual characteristics. We complement our qualitative scaling treatment with structure-based numerical calculations to rationalize each contribution to ion transport in the presence of strong interfacial effects.

P0.15 Supramolecular zippers of 10-N-nonyl acridine orange elicit interbilayer adhesion of membranes producing cell death

VÍCTOR G. ALMENDRO-VEDIA,^{1,§,§} Carolina García,² Diego de la Fuente-Herreruela,¹ Mónica Muñoz-Úbeda,³ Paolo Natale,¹ Rodrigo Queiroz-Albuquerque,⁴ Andrés Guerrero-Martínez,¹ Francisco Monroy,¹ Pilar Lillo,² Iván López-Montero.¹

¹Dto. Química Física I, Universidad Complutense de Madrid, Madrid, Spain; ²Dto. Química Física Biológica, Instituto de Química-Física "Rocasolano" (CSIC), Madrid, Spain; ³Instituto de Investigación Hospital Doce de Octubre (i+12), Madrid, Spain; ⁴Instituto de Investigación Hospital Doce de Octubre, Madrid, Spain.

§vgavedia@ucm.es, §*SBE bursary*

The fluorescent dye 10-N-nonyl acridine orange (NAO) is widely used as a mitochondrial marker. Very early, NAO was reported to have cytotoxic effects in cultured eukaryotic cells when incubated at high concentrations. Although the biochemical response of NAO-induced toxicity was well identified, the underlying molecular mechanism has not been yet explored in detail. Here, by using giant unilamellar vesicles and fluorescence confocal microscopy, we have obtained direct evidence that NAO promotes strong membrane adhesion of negatively charged vesicles. The attractive interactions between adhering membranes derive from van der Waals interactions between antiparallel H-dimers of NAO molecules from opposing bilayers, as revealed by fluorescence lifetime imaging microscopy. Semiempirical calculations have confirmed the supramolecular scenario by which antiparallel NAO molecules form a zipper of bonds at the contact region. The membrane remodelling effect of NAO, as well as the formation of H-dimers, was also confirmed in cultured fibroblasts, as shown by the ultrastructure alteration of the mitochondrial cristae. We conclude that membrane adhesion induced by NAO stacking accounts for the supramolecular basis of its cytotoxicity.

PO.16 Length-dependence of the elastic response of single-stranded DNA

XAVIER VIADER-GODOY,[§] Maria Manosas, Félix Ritort.

Departament de Física de la Matèria Condensada, Universitat de Barcelona, Barcelona, Spain.

[§]xaviviader@hotmail.com

Single-stranded DNA (ssDNA) plays a major role in several biological processes, such as replication or transcription. Therefore, it is of fundamental interest to understand the elastic response of this biological polymer. Besides, force spectroscopy techniques have been widely used to study biochemical and enzymatic processes involving DNA. The interpretation of the results obtained by these experiments requires an accurate description of the elastic properties of ssDNA. However, a large dispersion on the elastic parameters is obtained from different methods and sequences. In this work, we study the elastic properties of ssDNA using molecules with different sequences and lengths comprising 4 orders of magnitude (from 60bases to 14kbases). Using the inextensible Worm-Like Chain model we prove that the apparent discrepancy found in the previous works arises mainly from the different range of forces used to fit long and short molecules. We have also tested sequences with different pyrimidine/purine content in order to investigate the effect of base stacking, which is known to largely change the elastic properties of homogeneous sequences. Even that the stacking of bases has a minor impact in the elastic response of heterogeneous sequences, we detect base stacking effect at the level of tenths of bases.

PO.17 Base pair free energies derivation in RNA by single-molecule experiments

PAOLO RISSONE,[§] Cristiano Valim-Bizarro, Marco Ribezzi-Crivellari, Félix Ritort.

Small Biosystems Lab, Condensed Matter Physics Department, Universitat de Barcelona, Barcelona, Spain.

[§]rissone.paolo@gmail.com

Basic questions regarding the Folding/Unfolding process, fluctuation theorems, the thermodynamics and kinetics of small systems, and the physics of molecular motors were experimentally accessed using DNA and RNA hairpins specifically designed to these tasks. These DNA and RNA hairpins have been extensively used in the recent years as molecular tools in different single-molecule experiments. Thanks to these techniques it has been possible to obtain a direct measure [1] of the 10 free energy values (then reduced to 8 by introducing the circular symmetry) that mark the formation of a base pair in the DNA hairpin at different salt conditions. The following step consists in applying the same method in order to obtain the same univocal, single-molecule derived set of free energies for an RNA molecule. However, RNA physical and biological properties of RNAs strongly differ from DNAs one: the difficulties in the synthesis of long RNA hairpins suitable for the experimental setup and the presence of a strong hysteresis that affects the Unfolding/Refolding cycles, make the analysis of these molecules more complex than in the previous case. Here we present the on-going results obtained in the analysis of 2k basepairs RNA molecules and the techniques developed to overcome these difficulties.

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P0.18 Temperature-dependent of the secondary structure and the elastic response of RNA molecule.**J. SENDROS,^{1,§} A. Severino,¹ L. Asensio,¹ F. Ritort.^{1,2}**¹*Small Biosystems Lab, Universitat de Barcelona, Barcelona, Spain;* ²*CIBER-BBN, Instituto de Salud Carlos III, Madrid, Spain.*§ jsg.0711@gmail.com

RNA is one of the most important molecule involve in the synthesis of proteins. For this reason, the study and the knowledge of RNA elastic properties is fundamental to understand theses process. In this work has been studied the elastic response of RNA molecule at different temperatures by force spectroscopy techniques, where has been shown on one hand, the existence of an unexpected missfolded state at low temperatures, and on the other hand, the rejection Bell-Evans model due to the large hysteresis present in rupture forces of theses molecules. The formation of secondary structure has been analysed through the force-extension curves obtained, leading to the formation of the secondary structure depends on the temperatures.

P1 | Protein Structure, Dynamics and Function

Hall Posters and Exhibitors Hall

Odd-numbered posters

Date: Thursday, June 21st

Time: 19:00-20:00

Even-numbered posters

Date: Friday, June 22nd

Time: 12:00-13:00

P1.1 Structural analysis of the T7 bacteriophage infection machinery

ANA CUERVO,[§] Mar Pérez-Ruiz, José López-Carrascosa.

CNB-CSIC, Madrid, Spain.

[§]acuervo@cnb.csic.es

The bacterial envelope is the major barrier that bacteriophages have to overcome during infection. Most bacteriophages use a specialized complex, the tail, that serves as a conduit for genome transport. T7 infects *E. coli* bacteria and belongs to the *Podoviridae* family, which is characterized by a short non-contractile tail. The tail machinery is formed by the fibers, and the major tail proteins gp11 and gp12, that form a tubular channel for DNA transport. Unfortunately, T7 tail is too short to cover the complete width of the bacterial membrane. It has been shown that T7 needs an additional complex for DNA transport. This complex, named the core, is formed at least by two proteins (gp15 and gp16) and forms a tubular structure that enlarges the tail channel. We have performed the structural characterization of different ejection complexes using cryo-electron microscopy and image reconstruction methods. This technique, that has experienced an enormous improvement in the last years, is currently able to obtain near-atomic resolution structures by averaging thousands of electron microscope images. The multiple conformational states of the ejection complexes, together with their size and flexibility, makes these extremely hard to crystallize proteins, ideal candidates for their study using new cryo-EM facilities.

P1.2 Surface pressure effects on human MFN1-containing membrane monolayers

ANDRES DARIO TOLOSA-DIAZ,^{§,§}

Universidad Complutense de Madrid, Madrid, Spain.

[§]atolosa@ucm.es, [§]*SBE bursary*

Mitochondrial fusion and fission regulate mitochondrial homeostasis that is important for correct mitochondrial function and the energy balance of the eukaryotic cell. Protein-dependent mitochondrial fusion is a two-step process with the action of Mitofusins 1 and 2 (MFN1 and MFN2, respectively) that promote the fusion of the outer mitochondrial membrane and the Optic Atrophy 1 (OPA1) protein responsible for the fusion of inner mitochondrial membrane. The fusion event is catalyzed by hydrolysis of GTP that takes place in either a homo or hetero-oligomeric fashion. However, the detailed molecular mechanisms underlying the fusion event is not fully understood. Here, we have heterologously produced full length human MFN1 in *E. coli* SF100 (DE3) to explore the interfacial properties of Langmuir monolayers made from *E. coli* inner membrane embedding MFN1. A change in the lateral lipid monolayer pressure was observed when GTP and Mg²⁺ were supplied to the MFN1 containing lipid monolayer. This packing effect was not observed for *E. coli* membranes monolayers in the absence of MFN1. A change in lipid packaging suggests a reorganization of the oligomeric state of MFN1 upon the binding of GTP. This change might prime the MFN1 for fusion with a complement set of membranes containing MFN1 or MFN2.

P1.3 Study of the antioxidant activity of human and yeast frataxin

BARTOLOMÉ VILANOVA-CANET,[§] Ana B. Uceda, Juan Frau, Josefa L. Donoso, Laura Mariño, Miquel Adrover.

University of Balearic Islands, Mallorca, Spain.

[§]bartomeu.vilanova@uib.es

Human frataxin is a mitochondrial protein that in humans is encoded by so-called FXN gene. Despite the fact that the role of the protein is not fully understood yet, it is well known that its deficiency leads to intracellular accumulation of iron and to an increase in the levels of reactive oxygen species (ROS), causing improper function of the mitochondria and cellular metabolism in general. ¹ In fact, low levels of frataxin expression give rise to a neurodegenerative disease known as Friedreich's ataxia,² in which oxidative stress plays a major role. ³ Here, we have used a broad range of spectroscopic techniques to study the capacity of human (Hfra) and yeast (Yfh1) frataxin to scavenge or inhibit the formation of ROS. Overall, the obtained results reveal that both frataxins possess the same capacity to inhibit the formation of HO• and to scavenge O₂•⁻. On the other hand, Yfh1 slows down the formation of H₂O₂. Moreover, Hfra is less susceptible to HO• damage than Yfh1, suggesting that evolution has made Hfra less prone to oxidative damage than Yfh1.

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P1.4 Supramodular dynamics regulated by ligand binding in PDZ tandem

BERTALAN KOVÁCS,[§] Zoltán Gáspári.

Faculty of Information Technology and Bionics, Pázmány Péter Catholic University, Budapest, Hungary.

[§]kovacs.bertalan@itk.ppke.hu

PDZ domains are common protein-binding modules, recognizing short C-terminal sequences. In the tandem arrangement, PDZ domains usually fold and in an interdependent way, and they need sequential proximity in order to exert proper biological function. The PDZ1-2 tandem of the PSD-95 protein was shown to possess a rigid structure in the apo-state, however ligand binding induces considerable interdomain mobility. We presume that regulation of the relative orientation and dynamics of the two PDZ domains might be a key feature in organizing binding partners of PSD-95, and, eventually, clustering membrane receptors in the post-synaptic site of nerve cells. In order to elucidate the atomic-level mechanism of the inner motions in the PDZ tandem, we performed all-atom molecular dynamics (MD) simulations, including NMR-derived experimental dynamic parameters (NOEs and S2 order parameters) as external restraints. As a result, we obtained a number of dynamic structural ensembles of the modeled structure that properly describe its dynamics on the fast (ps-ns) timescale while maintaining good correspondence with the observable dynamic parameters. Analysis of the structural ensembles shed light on the role of the β 2- β 3 loop in the PDZ domains as the key to ligand-induced dynamics and interdomain communication.

P1.5 Orthogonal fingerprinting for accurate and fast single-molecule mechanical profiling of proteins

CARMEN SUAY-CORREDERA,^{1,§} Carolina Pimenta-Lopes,² Diana Velázquez-Carreras,¹ David Sánchez-Ortiz,¹ Jorge Alegre-Cebollada.¹

¹*Spanish National Center for Cardiovascular Research (CNIC), Madrid, Spain;* ²*Department of Physiological Sciences, University of Barcelona, Barcelona, Spain.*

[§]csuay@cnic.es

Force-spectroscopy by Atomic Force Microscopy (AFM) is the gold-standard technique to characterize the nanomechanics of proteins and their interactions with ligands. A well-recognized shortcoming of AFM stems from a 15-20% calibration uncertainty, which leads to interexperimental variations in force that limit the accuracy of mechanical profiling of proteins. Here, we develop orthogonal fingerprinting strategies to track mechanical unfolding of two different proteins in the same AFM experiment, under the same force calibration. We combine single-molecule force-spectroscopy, theoretical modelling of error propagation, and Monte Carlo simulations to show that the accuracy of simultaneous measurement by orthogonal fingerprinting is independent of calibration uncertainty, reaching up to a 6 fold improvement with respect to traditional AFM. This improvement in accuracy is preserved even when unfolding force data are obtained from multiple, independent orthogonal fingerprinting experiments. We also demonstrate that orthogonal fingerprinting can speed up data acquisition more than 30 times to reach the same accuracy as traditional AFM. Orthogonal fingerprinting further reduces experimentation time by simultaneous purification of proteins. Benefiting from the increased accuracy of orthogonal fingerprinting, we validate the common assumption that the mechanical stability of a protein is independent of its neighboring domains

P1.6 Native redox posttranslational modifications as regulators of titin mechanical properties

ELÍAS HERRERO-GALÁN,[§] Cristina Sánchez-González, Diana Velázquez-Carreras, Elena Bonzón-Kulichenko, Enrique Calvo, Jesús Vázquez, Jorge Alegre-Cebollada.

CNIC, Madrid, Spain.

[§]elias.herrero@cnic.es

The relevant role of titin in the contractility of cardiac muscle has been recently evidenced by the discovery of mutations in the titin gene that lead to pathologies involving changes in the elasticity of the heart. The elastic properties of titin depend on the folding/unfolding equilibria of its immunoglobulin (Ig) domains. Strain-induced posttranslational modifications of buried cysteines have been recently shown to be key regulators of the folding dynamics of titin Ig domains. However, the identity, extent and specific residues targeted by these modifications *in vivo* remain unexplored. We have optimized a method for in-gel detection of oxidized thiols by fluorescent labeling, which has allowed us to provide the first experimental evidence that a significant fraction of titin's cysteines are oxidized. By mass spectrometry and high resolution structure modelling we study the redox state of specific cysteine residues and predict the presence of disulfides in the different Ig domains of titin. Furthermore, by means of single-molecule atomic force spectroscopy, we aim to determine the effect of these modifications in the elasticity of the protein. This approach may lead to a better understanding of how the contractility of the heart is modulated in physiology and disease.

P1.7 Current possibilities and upgrades of xaloc, the MX beamline at ALBA synchrotron

FERNANDO GIL,[§] Barbara Calisto, Xavier Carpena, Nahikari González, Jordi Andreu, Ricardo Valcárcer, José Avila, Jorge Villanueva, Judith Juanhuix, Roeland Boer.

ALBA Synchrotron, Cerdanyola del Vallés, Spain.

[§]fgil@cells.es

BL13-XALOC is a tunable MX beamline at the 3rd generation synchrotron ALBA (Barcelona), in user operation since 2012. XALOC has been designed to deal not only with easily automatable X-ray diffraction experiments of micrometer-sized crystals, but also with more complex ones including a variety of crystal sizes, unit-cell dimensions and crystals with high mosaic spread and/or poor diffraction. The aim for a reliable all-in-one beamline is equaled by the aim to maximize ease-of-use and automatization. Remote data collection is supported covering the dewar transport expenses. The beamline allows “in-situ” diffraction. Fast automatic data processing (autoPROC) and a new pipeline for data reprocessing/crystal structure determination (Xamurai) are available. MXCube and ISPyB for data collection and sample tracking/experiment reporting are implemented already. The CATS sample changer now supports both EMBL/ESRF and Uni-puck standards with a capacity of up to 108 samples. To boost data collection at long X-ray wavelengths a Helium chamber has been developed. Continuous access to the beamline is available, allowing access within a few weeks. Current possibilities of the beamline and upgrades that will become available in the near future will be discussed. In addition, the MX microfocus beamline XAIRA, currently in the design phase, will be introduced briefly.

P1.8 Structural basis and energy landscape for the Ca²⁺-gating and calmodulation of the K_v7.2 K⁺ channel

GANEKO BERNARDO-SEISDEDOS,^{1,§} Carolina Gomis-Pérez,¹ Covadonga Malo,¹ Alvaro Villarroel,¹ Óscar Millet.²

¹*Instituto Biofisika, Leioa, Spain;* ²*CIC bioGUNE, Leioa, Spain.*

[§gbernardo.atlas@cicbiogune.es](mailto:gbernardo.atlas@cicbiogune.es)

K_v7.2 (KCNQ2) channel is the principal molecular component of the slow voltage-gated non-inactivating K⁺ M-current, a key controller of the neuronal excitability. To investigate the calmodulin-mediated Ca²⁺ gating of the channel, we used NMR spectroscopy to structurally and dynamically describe the association of helices *hA* and *hB* of K_v7.2 with calmodulin (CaM), as a function of Ca²⁺ concentration. The structures of the CaM/K_v7.2-hAB complex at three different calcification states are here reported. In the presence of a basal cytosolic Ca²⁺ concentration (10-100 nM) only the N-lobe of calmodulin is Ca²⁺-loaded and the complex (representative for the open channel) exhibits collective dynamics in the millisecond timescale towards a lowly populated excited state (1.5%) that corresponds to the inactive state of the channel. In response to a chemical or electrical signal, intracellular Ca²⁺ levels rise up to 1-10 μM, triggering Ca²⁺ association to the C-lobe. The associated conformational rearrangement is the key biological signal that shifts populations to the closed/inactive channel. This reorientation affects C-lobe of CaM and both helices in K_v7.2, allosterically transducing the information from the Ca²⁺ binding site to the trans-membrane region of the channel.

P1.9 Drug discovery with a fret-biosensor of scaffolding interactions in the N-terminal region of c-Src

GUILLERMO IRUELA-MARTÍN,^{1,2,§,§} Miguel Arbesú,¹ Joao M. C. Teixeira,¹ Miquel Pons.¹

¹*University of Barcelona, Barcelona, Spain;* ²*IDP Discovery Pharma, Barcelona, Spain.*

[§giruela20@gmail.com](mailto:giruela20@gmail.com), [§ ISMAR bursary](#)

The non-receptor tyrosine kinase c-Src is involved in numerous signal transduction pathways and high levels of c-Src activity have been associated with a poor prognosis in colorectal, prostate, and breast cancers¹. In the last decades, structural and biochemical studies have shown the crucial role of the Unique domain in the regulation of c-Src activity, additionally, mutations on this domain cause strong phenotypes in the maturation of *Xenopus laevis* oocytes². The Unique and SH4 domains are intrinsically disordered regions (IDR) of c-Src, attached to the globular SH3 domain. This N-Terminal region of c-Src adopt a compact, yet highly dynamic structure that can be described as an intramolecular fuzzy complex³. So far, the cancer therapeutical drugs have been targeted to the kinase domain (SH1) of c-Src, arising the problem of non selective interaction with other kinases. Targeting the N-terminal region containing the unique domain would provide a higher selectivity to the lead. The design and characterization of a new FRET-biosensor involving the N-Terminal regulatory region of c-Src show a new perspective to find new leads.

P1.10 Cytochrome c speeds up caspase cascade activation by blocking 14-3-3 ϵ -dependent apaf-1 inhibition

IRENE DIAZ-MORENO,^{1,§} Carlos A. Elena-Real,¹ Antonio Díaz-Quintana,¹ Katiuska González-Arzola,¹ Adrián Velázquez-Campoy,² Mar Orzáez,³ Abelardo López-Rivas,⁴ Miguel A. de la Rosa.¹

¹Institute for Chemical Research (IIQ - cicCartuja), Sevilla, Spain; ²Institute of Biocomputation and Physics of Complex Systems (BIFI), Joint Unit IQFR-CSIC-BIFI, Zaragoza, Spain.; ³Centro de Investigación Príncipe Felipe, Valencia, Spain; ⁴Centro Andaluz de Biología Molecular y Medicina Regenerativa-CABIMER, Sevilla, Spain.

§idiazmoreno@us.es

Apoptosis is a highly regulated form of programmed cell death, essential to the development and homeostasis of multicellular organisms. Cytochrome c is a central figure in the activation of the apoptotic intrinsic pathway, thereby activating the caspase cascade through its interaction with Apaf-1. Our recent studies have revealed 14-3-3 ϵ (a direct inhibitor of Apaf-1) as a cytosolic cytochrome c target. Here we explore the cytochrome c / 14-3-3 ϵ interaction and show the ability of cytochrome c to block 14-3-3 ϵ -mediated Apaf-1 inhibition, thereby unveiling a novel function for cytochrome c as an indirect activator of caspase-9/3. We have used calorimetry, NMR spectroscopy, site mutagenesis and computational calculations to provide an insight into the structural features of the cytochrome c / 14-3-3 ϵ complex. Overall, these findings suggest an additional cytochrome c-mediated mechanism to modulate apoptosome formation, shedding light onto the rigorous apoptotic regulation network.

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P1.11 Self-association of the n-terminal regulatory region of c-Src

MOHAMMAD-JABEEN IRREM-LAAREB,^{§,§} Anabel Lise Le Roux, Miguel Arbesú, Miquel Pons.

Biomolecular NMR Laboratory, Department of Inorganic and Organic Chemistry, University of Barcelona, Barcelona, Spain.

§laareb.mohammad@ub.edu, § *ISMAR bursary*

The N-terminal regulatory region of the non-receptor protein kinase c-Src including the myristoylated SH4, Unique and SH3 domains (USH3), anchors the protein to the inner surface of the cell membrane. The insertion of the myristoyl chain and the electrostatic interaction of the positively charged SH4 domain residues contribute to this interaction. High levels of c-Src activity have been related to several human cancers. The binding of c-Src to the lipid membrane, is labile and the interaction easily reversible. However, we identified a small population of c-Src, formed by dimers or trimers, that remains persistently attached to lipid membranes. Self-association of the isolated myristoylated SH4 domain (residues 2-17) has also been observed and leads to the formation of large clusters, when the domain is directly fused to reporter proteins. The neighbor Src domains, seem to play a role in restricting the oligomerization to discrete dimers or trimers. We use Site Directed Mutagenesis and Surface Plasmon Resonance to investigate the structural determinants of c-Src self-association on lipid membranes.

P1.12 Understanding the structural origins of α -synuclein fibril polymorphism

JOSÉ DANIEL CAMINO-CAMINO,^{1,§,§} Serene W. Chen-Chen,² Jesús Sot,³ Igor de la Arada,³ José L. Arrondo,³ Félix M. Goñi,³ Christopher M. Dobson,² Nunilo Cremades.¹

¹*Institute for Biocomputation and Physics of Complex Systems (BIFI), University of Zaragoza, Zaragoza, Spain;*

²*Department of Chemistry, University of Cambridge, Cambridge, United Kingdom;* ³*Department of Biochemistry, University of Basque Country, Leioa, Spain.*

§ jdcamino@bifi.es, § *SBE bursary*

α -Synuclein (α -Syn) is a presynaptic intrinsically disordered neuronal protein whose misfolding and aggregation in the form of amyloid fibrils is the hallmark of a range of neurodegenerative disorders, including Parkinson's disease, that are collectively referred to as synucleinopathies. Recent experimental evidences suggest that different types of fibrillar polymorphs of α -Syn present different degrees of infectivity, suggesting a potential link between the structure and pathology of different α -Syn fibrillar polymorphs. Indeed, the ability of α -Syn to aggregate in different amyloid polymorphs has been associated with its ability to cause different synucleinopathies, suggesting a potential role of distinct structural types of α -Syn aggregates in the development of specific types of synucleinopathies. With the aim of understanding the structural origins of α -Syn fibril polymorphism, we have generated a battery of different α -Syn fibril polymorphs and have characterised their morphological and structural features with the final goal of relating their structural features with their ability to induce neuronal toxicity and infectivity.

P1.13 Protein-protein contacts enhance amyloidogenesis. An infrared spectroscopy study

JOSÉ LUIS RODRIGUEZ-ARRONDO.[§]

UPV/EHU, Bilbao, Spain.

§ joseluis.arrondo@ehu.eus

Protein misfolding, which includes the formation of amyloid aggregates is related to a large number of different diseases, most of them neurodegenerative. Hen Egg White Lysozyme (HEWL) has often been used as an amyloid model because it is a good characterized protein with ability to form these aggregates when exposed to extreme conditions. Usually, the "in vitro" amyloid formation studies are done in a medium which can be considered as diluted in number of particles present, and the process of aggregation in the test tube may differ from the one inside the cell, mainly because its internal concentration is crowded with numerous macromolecules. We have studied the process of amyloid formation in HEWL in the presence of aggregating agents mimicking a crowded environment. Aggregating agents Dextran 40, Dextran 70 and Ficoll 70 at concentrations of 5%, 10% and 20% have been used. One- and two- dimensional Infrared (IR) Spectroscopy have been used in order to characterise the process of amyloid formation. It is shown that lysozyme aggregates are not formed in the same way in a diluted environment or in a crowded one.

P1.14 Structural insights in gene expression modulation by response regulator RcsB

JUAN JOSÉ HUESA,^{§,§} Patricia Casino.

Universitat de València, Valencia, Spain.

[§]juan.huesa@uv.es, [§] *SBE bursary*

Rcs phosphorelay system is a complex signal transduction system present in *Enterobacteriaceae*, a large Gram-negative bacteria family. The Rcs phosphorelay system can sense different signals that cause perturbation of the cell envelope and regulate different genes related with meta-bolism, cell division and motility in order to adapt to the stressed environment. The Rcs phosphorelay system is composed basically by three proteins, the hybrid histidine kinase RcsC that autophosphorylates upon sensing a signal, the phosphotransferase RcsD that accepts a phosphoryl group from RcsC and transfers it to RcsB, which is a response regulator that acts as a transcriptional factor modulating gene expression by activating or inhibiting transcription. RcsB can regulate genes either alone, as a homodimer, or together with auxiliary transcriptional factors, as a heterodimer, a role that can be dependent or independent of phosphorylation. To shed light on the RcsB gene transcription regulation mechanism through phosphorylation we have solved the crystal structure of RcsB in the presence of the *bona fide* promoter $P1_{flhDC}$ in the *flhDC* operon which demonstrates the RcsB homodimerization dependent on phosphorylation for DNA binding. Furthermore, we have also analyzed the influence of specific residues related with the switch between non-phosphorylation and phosphorylation conformations through functional studies.

P1.15 Supramolecular structure regulate β -Gal functionality

JULIETA MARÍA SANCHEZ,[§] Sandra Soledad Flores, Eduardo Matias Clop, Pedro Diego Clop, María Verónica Nolan, María Angélica Perillo.

IIByT, CONICET e ICTA, FCEFyN. UNC. Córdoba, Argentina.

[§]jsanchezqa@gmail.com

In our laboratory we studied the structure-function relationship of proteins in complex environment. We applied β -galactosidase from *E. coli* (β -Gal) as a model protein. Previously we proved that the soluble enzyme interacts with lipid/water interfaces and demonstrated that in this condition β -Gal activity increased and acquired a structure more hydrated and more resistant to thermal unfolding with respect to β -Gal in solution. We also prepared mixed β -Gal/phospholipid Langmuir films (LF) at the air/water interface. At high surface pressures the AFM analysis of LF suggested that β -Gal was in a state of higher order of oligomerization than the typical tetramer. Currently, we overexpress a recombinant β -Gal in *E. coli*. In some conditions we obtain β -Gal inclusion bodies (IBs $_{\beta$ -Gal}). Interestingly, the IBs $_{\beta$ -Gal} exhibits not only higher activity but also higher resistance to temperature and pH inactivation with respect to the soluble β -Gal. We conclude that the protein-protein contact at the lipid/water and at the protein/water interfaces confers β -Gal an activating environment allowing a non-native but active and stable structure.

P1.16 Coordinated activity of the human mitochondrial DNA helicase (TWINKLE) with SSB proteins and the human mitochondrial DNA polymerase

KATERYNA LEMISHKO,^{1,§} Grzegorz L. Ciesielski,² José López-Carrascosa,³ Laurie S. Kaguni,² Borja Ibarra.²

¹Imdea Nanoscience, Madrid, Spain; ²Spanish National Center for Biotechnology (CNB-SCIC), Madrid, Spain; ³Department of Biochemistry and Molecular Biology, Michigan State University, Michigan, United States; ⁴Institute of Bioscience and Medical Technology, University of Tampere, Tampere, Finland..

§kate.lemishko@gmail.com

Mitochondria are eukaryotic organelles, responsible for cellular energy generation. Besides their role in energy production, mitochondria are essential for cellular activity regulation, e.g. cell signaling and cell death. Mitochondria have their own DNA (mtDNA) and, in general, human cells contain thousands of mtDNA copies. A reduction in the number of mitochondrial DNA molecules or accumulation of mtDNA mutations may cause so-called ‘mitochondrial diseases’. Therefore, mitochondrial dysfunction in some measure depend on effectiveness and accuracy of mtDNA replication. To understand the molecular basis of these diseases, it is important to define the molecular mechanisms that govern the activity of the proteins participating in human mitochondrial DNA replication. The ‘minimal replisome’, that is capable of processive DNA synthesis, can be reconstituted in vitro with just three proteins: the TWINKLE DNA helicase, the single-stranded DNA binding protein (mtSSB) and the mitochondrial DNA polymerase gamma. In this work we used optical tweezers technique to 1) study the activity of individual TWINKLE molecules at the replication fork, 2) to determine the effect of human mitochondrial single stranded DNA binding protein (mtSSB) on the TWINKLE helicase activity, 3) to study the influence of the TWINKLE helicase on the polymerase gamma kinetics during strand displacement DNA synthesis.

P1.17 Combining structural aggregation propensity and stability predictions to re-design protein solubility

MARCOS GIL-GARCÍA,^{1,§,§} Nathalia Varejão,¹ Michal Jamroz,² Aleksander Kuriata,² Marta Diaz-Caballero,¹ Jara Lascorz,¹ Susanna Navarro,¹ David Reverter,¹ Sebastian Kmiecik,² Salvador Ventura.¹

¹Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallés, Spain; ²Biological and Chemical Research Centre, Faculty of Chemistry, University of Warsaw, Warsaw, Poland.

§marcosgil94@gmail.com, §*SBE bursary*

The aggregation propensity of each particular protein seems to be shaped by evolution according to its natural abundance in the cell. The production and downstream processing of recombinant polypeptides implies attaining concentrations that are orders of magnitude above their natural levels, often resulting in their aggregation; a phenomenon that precludes the marketing of many globular proteins for biomedical or biotechnological applications. Therefore, there is a huge interest in methods aimed to rise proteins solubility above their natural limits. Here, we demonstrate that an updated version of our AGGRESKAN 3D structural aggregation predictor, that now takes into account protein stability, allows designing mutations at specific positions in the structure that improve the solubility of proteins without compromising their conformation. Using this approach, we have designed a highly soluble variant of the Green Fluorescent Protein (GFP) and a human single-domain VH antibody displaying significantly reduced aggregation propensity. Overall, our data indicate that the solubility of unrelated proteins can be easily tuned by in silico-designed non-destabilizing amino acid changes at their surfaces.

P1.18 Thermodynamic impact of the I603M mutation in cardiac myosin binding protein C: A potential role in the development of hypertrophic cardiomyopathy

MARIA ROSARIA PRICOLO,^{1,§,§} Carmen Suay-Corredera,² Diana Velázquez-Carreras,² Elías Herrero-Galán,² Giulia Frisso,³ Jorge Alegre-Cebollada.²

¹Università di Napoli “FedericoII” and Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; ²Centro Nacional de Investigaciones Cardiovasculares Carlos III (CNIC), Madrid, Spain; ³Università di Napoli “FedericoII”, Napoli, Italy.

§mariarosaria.pricolo@unina.it, §SBE bursary

Hypertrophic cardiomyopathy (HCM) is the most common genetic disease of the myocardium. In ~60% of the cases HCM is caused by mutations in sarcomeric proteins, such as Myosin Binding Protein C (cMyBPC), which are responsible for generating the molecular force of myocyte contraction. We have screened a cohort of HCM patients for mutations in sarcomeric genes, and we have found a new variant of cMyBPC, I603M, which we have selected for functional study to determine pathogenicity. This mutation is localized in domain C4 of cMyBPC. Using bioinformatics sequence analyses we predict a deleterious effect for I603M, but mRNA studies do not show any alteration of the splicing mechanism. At the protein level, homology modelling of domain C4 shows I603 to be buried in the protein structure, suggesting a potential destabilizing role of the I603M mutant. Indeed, circular dichroism spectroscopy shows a lower melting temperature for the mutant C4 domain (42 °CC as opposed to 57 °CC for the WT). Results obtained by single-molecule atomic force microscopy do not show a mechanical fingerprint for either WT or the mutant C4 domain. We propose that mutation I603M leads to a strong thermal destabilization of the domain, altering the function of cMyBPC by protein haploinsufficiency.

P1.19 A surface to twist the FtsZ filament: A good strategy to generate force

MARISELA VELEZ-TIRADO,^{1,§} Ileana Márquez,¹ Pedro Tarazona.²

¹ICP-CSIC, Madrid, Spain; ²Universidad Autonoma de Madrid, Madrid, Spain.

§marisela.velez@icp.csic.es

FtsZ is a bacterial cytoskeletal protein that polymerizes on the inner surface of the bacterial membrane and contributes to generate the force needed for cell division. In the presence of GTP the individual protein monomers interact longitudinally to form filaments that can then aggregate to form higher order structures on the membrane surface. These filament aggregates are dynamic and exchange monomers from the solution. The outcome of this dynamic rearrangement on the surface is the generation of force that bends the cell membrane inward. The rich self-assembling behavior of the protein is reproduced *in vitro* on supported lipid membranes using isolated proteins. Atomic Force Microscopy (AFM) reveals detailed structural and dynamic information of single filaments and allowed, for the first time, to compare single filament experimental results with theoretical models that describe the polymerization in terms of a simple set of monomer-monomer interactions. Experimental results controlling the orientation of the monomers on the surface, together with molecular dynamics simulations and theoretical models have revealed that, unexpectedly, filament curvature, twist, orientation and the strength of the surface attachment are all important for determining the amount of force that the filaments can exert on the surface.

P1.20 Structural and biochemical characterization of a ferredoxin-NADP⁺ reductase from the brucella ovis pathogen

MARTA MARTÍNEZ-JÚLVEZ,^{1,§} Víctor Taleb,² Daniel Pérez-Amigot,² Ernesto Anoz-Carbonell,¹ María Sebastián,¹ Sergio Boneta,³ Víctor Polo,⁴ Milagros Medina.¹

¹Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias and Instituto de Biocomputación y Física de Sistemas Complejos (BIFI) and GBsC-CSIC and BIFI-CSIC Joint Units, Universidad de Zaragoza, Zaragoza, Spain; ²Departamento de Bioquímica y Biología Molecular y Celular, Facultad de Ciencias, Universidad de Zaragoza, Zaragoza, Spain; ³Departamento de Química Física, Universidad de Zaragoza, Zaragoza, Spain; ⁴Departamento de Química Física and Instituto de Biocomputación y Física de Sistemas Complejos (BIFI) and GBsC-CSIC and BIFI-CSIC Joint Units, Universidad de Zaragoza, Zaragoza, Spain.

§ mmartine@unizar.es

Brucella ovis is a gram-negative coccobacillus that causes ovine brucellosis, a clinical or subclinical disease characterized by genital lesions in rams and placentitis in female sheep. The infection is transmitted congenitally to their offspring, resulting in fertility loss and an increase in perinatal mortality [1]. The *Brucella ovis* proteome contains a FAD containing Ferredoxin-NADP(H) reductase (FPR) that belongs to the bacterial subclass 1 FPR family, (*BoFPR*). By similarity with family members *BoFPR* is expected to deliver electrons from NADPH, or low potential one-electron donors, to the redox-based metabolism. Due to its potentially relevant role for the pathogen survival upon under infection conditions, it might be a potential drug target. We have overproduced *BoFPR*, presenting here its first biochemical and structural characterization, including resolution of its three dimensional structure at 1.69 Å and kinetic characterization of its reduction by the NADPH coenzyme. Moreover, all-atom molecular dynamics simulations are also used to evaluate protein dynamics and to predict the location of the nicotinamide moiety of NADPH in a catalytically competent conformation.

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P1.21 Discriminating the effect of charged metabolite interactions on protein structure and stability

MICAEL SIMÕES-SILVA,^{1,§,§} Aldino Viegas,¹ Angelo M. Figueiredo,² Eurico Cabrita.¹

¹Faculdade de Ciências e Tecnologia - Universidade Nova de Lisboa, Portugal; ²University College London, London, United Kingdom.

§ msi.silva@campus.fct.unl.pt, § *ISMAR bursary*

Different environmental factors can change the composition of the protein environment and have been associated with protein misfolding and, ultimately, with neurodegenerative diseases such as Alzheimer's or Parkinson's. One very important aspect is that the intracellular environment is extremely crowded with small charged metabolites that can form ion-pairs and have the potential to act on the folding and stability of proteins. Inspired by the high concentrations of organic charged metabolites found in the cell milieu, specifically the choline cation and the glutamate anion, and in our previous studies with imidazolium-based ionic liquids (IL), that disclosed the effects of specific P-IL interactions on protein stability, we studied the effect of the biocompatible IL [Ch][Glu] on the stability of the domain B1 of protein G (GB1), a globular and highly stable protein. In this communication, through the combination of different NMR techniques and the determination of protein stability by fluorescence and calorimetric studies, we will present results concerning the discrimination of the nature of the contacts established by ion-pairs and the ions alone. We expect to contribute to an understanding of how changes in the cellular homeostasis may control the protein folding landscape.

P1.22 Conformation-function relationships in the human apoptosis inducing factor family

MILAGROS MEDINA,[§] Raquel Villanueva, Ruben Laplaza, Silvia Romero-Tamayo, Nerea Novo, José Alberto Carrodeguas, Patricia Ferreira-Neila.

Departamento de Bioquímica y Biología Molecular y Celular y BIFI. Universidad de Zaragoza, Zaragoza, Spain.

[§mmolina@unizar.es](mailto:mmolina@unizar.es)

Apoptosis inducing factor isoform 1 (AIF1) is involved in the biogenesis of mitochondrial respiratory complexes and induces caspase-independent apoptosis [1], and categorized within a large family of eukaryotic proteins found in animals and fungi that also contains the AIF-like protein (AIFL or AIF3). AIFL, on its side, modulates mitochondrial morphology [2], induces apoptosis in a caspase-dependent manner [3], and is aberrantly expressed in mitochondria of cholangiocarcinoma patients (potential target for chemotherapy) [4]. Here, using spectroscopic, thermal denaturation and molecular simulations tools, we evaluate the effect of the R201 deletion, causative of the Cowchock syndrome in humans, on AIF1 conformation [5]. In addition, considering that hAIFL has neither been *in vitro* structurally nor functionally characterized, we show here an *in-silico* analysis that confirms it consists of an N-terminal Rieske and a pyridine nucleotide-oxidoreductase domain. This finding allowed us to build a hAIFL 3D homology model that might explain electron transfer between its redox cofactors.

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P1.23 Production of human pulmosaposin B1, the N-terminal saposin of the surfactant protein B precursor, in yeast: An improved system and a deeper functional characterization.

MIRIAM ISASI-CAMPILLO,^{1,§} M^a Fátima Lasala,² Ángeles Meléndez,² Fernando Chaves,² Rafael Delgado,² Jesús Pérez-Gil,¹ Lucía García-Ortega.¹

¹*Department of Biochemistry and Molecular Biology, Universidad Complutense, Madrid, Spain;* ²*Department of Microbiology and Instituto de Investigación Hospital Universitario 12 Octubre, Madrid, Spain.*

[§miriisacam@hotmail.com](mailto:miriisacam@hotmail.com)

Surfactant protein B (SP-B), from the saposin-like family (SAPLIP), is essential for lung function. Saposins are small proteins with a helical-based fold and the ability to interact with lipids. SP-B is synthesized *in vivo* as a larger precursor, proSP-B, with the mature protein flanked by N-terminal and C-terminal pro-peptides. proSP-B contains two additional saposins within the N-terminal and C-terminal modules, respectively. The N-terminal saposin (SP-B^N, or pulmosaposin B1) has been recently purified and associated with antimicrobial properties, suggesting a role in innate host defense. However, the recombinant protein employed in those studies was reconstituted from inclusion bodies in *E. coli*, contaminated with endotoxin and lacked glycosylation. To overcome this, validate previous results and perform more exhaustive structural and functional assays, we describe here the recombinant expression in *Pichia pastoris* of glycosylated and non-glycosylated human SP-B^N. Structural characterization by far-UV CD and analytical ultracentrifugation reveals high thermostability, a saposin-fold and dimerization ability. SP-B^N does not show stable interaction with lipid membranes although it leaks phosphatidylglycerol vesicles at low pH, with higher activity of the glycosylated form. This improved SP-B^N does not show remarkable bactericidal properties. Instead, pulmosaposin B1 prevents cell infection of enveloped viruses using a pseudotype model.

P1.24 Thiol-based redox systems: Diversity of thioredoxin reductases

MONICA BALSERA-DIEGUEZ,^{1,§} Rubén M. Buey,² Peter Schürmann,³ Bob B. Buchanan.⁴

¹IRNASA-CSIC, Salamanca, Spain; ²Universidad de Salamanca, Salamanca, Spain; ³Université de Neuchâtel, Neuchâtel, Switzerland; ⁴University of California, Berkeley, USA.

§monica.balsera@csic.es

Thioredoxins (Trxs) are key components of the redox system that regulates the activity of multiple target proteins through dithiol-disulfide exchange reactions. Trxs are reduced by members of the Trx reductase (TR) family that is composed of pyridine nucleotide-dependent flavoenzymes (NTRs) and ferredoxin- (Fdx)-linked iron-sulfur proteins (FTRs). NTRs, the most common type, are members of the family of dimeric pyridine nucleotide disulfide oxidoreductase flavoproteins that typically use NADPH as the source of reducing equivalents. Although NADPH is considered the primary source of reducing equivalents for the flavoprotein-linked Trx system, three decades ago a ferredoxin-linked flavoenzyme was described to function for the reduction of Trx in clostridia (Hammel and Buchanan, *Proc Natl Acad Sci U S A*, 1983). More recently, the list of NADPH-independent TR members has grown to include four previously unrecognized groups of flavoenzymes in bacteria and archaea. We plan to present recent findings on the structural characterization of this emerging family of flavoenzymes and demonstrate how their functional diversity is giving insight into the evolution of redox systems.

P1.25 Characterization of photoswitchable hydrophobic and amphiphilic peptides for folding and membrane insertion studies

MÓNICA VICTORIA GUTIÉRREZ-SALAZAR,^{1,§} Eduardo Santamaría,² Diego Sampedro,² Víctor A. Lórenz-Fonfría.¹

¹Instituto de Ciencia Molecular, Universidad de Valencia, Spain; ²Centro de Investigación en Síntesis Química, Universidad de La Rioja, Spain..

§mogusa@alumni.uv.es

The mechanism and kinetics of folding and insertion of hydrophobic and amphiphilic peptides in lipidic membranes is a process not fully understood. This is mainly due to the lack of experimental procedures that allow to follow the process with sufficient temporal and structural resolution. An approach to achieve this sensitivity is photocontrol of folding, using molecular photoswitches linked to the peptide. These photoswitches isomerize when irradiated with light of specific wavelengths, and can induce changes in the structure of the peptide, as folding or unfolding of the peptide and, consequently, modulate the insertion in the membrane. This work aims to characterize two model peptides (hydrophobic and amphiphilic) linked to a derivate of the photoswitch azobenzene (BCA), by circular dichroism, ultraviolet/visible and infrared spectroscopies, to use them in future folding and membrane insertion studies. We designed different lighting setups to achieve photoisomerization with precision. The photoswitchable peptides were characterized in membrane-mimicking environments and solvents to elucidate how differences in the environment modulate the peptide response to the isomerization of the photoswitch and the photochemistry of the photoswitch itself. Both hydrophobic and amphiphilic peptides showed good photoisomerization of BCA, and some promising results were obtained in the photocontrol of their structure.

P1.26 Linear motifs and a linker with multiple properties determine displacement ability on a multisite intrinsically disordered viral oncoprotein

NICOLÁS SEBASTIÁN GONZÁLEZ-FOUTEL,^{1,§,§} Wade Borchers,² Juliana Glavina,³ Ignacio E. Sánchez,³ Gonzalo de Prat-Gay,⁴ Gary Daughdrill,² Lucía B. Chemes.¹

¹Protein Structure-Plasticity and Function Laboratory. Institute of Biotechnological Investigations (IIB-CONICET). San Martín, Argentina; ²Center for Drug Discovery and Innovation. University of South Florida. Tampa, USA; ³Protein Physiology Laboratory (IQUIBICEN-CONICET) and School of Exact and Natural Sciences, University of Buenos Aires. Buenos Aires, Argentina; ⁴Protein Structure-Function Laboratory. Leloir Institute (IIBBA-CONICET). Buenos Aires, Argentina.

§nicolasgfoutel@gmail.com, §Best poster prize, ISMAR bursary

In this work we explored the biophysical basis for virus-host protein-protein interactions by studying the interaction between the human Adenovirus E1A protein (AdE1A) and the Retinoblastoma tumor suppressor protein (Rb). This interaction is mediated by two highly conserved linear motifs within AdE1A (E2F-like and LxCxE motifs), which are separated by a 70-residue “linker”. AdE1A is an intrinsically disordered monomer with extended hydrodynamic radius (Far UV-CD, SEC-SLS). Interaction assays show that AdE1A binds to Rb with 1:1 stoichiometry and an affinity $K_D = 24$ pM, accompanied by its strong compaction during complex formation. In addition, even though the individual sites bind Rb with lower affinity ($K_D = 110$ nM) than the cellular E2F counterpart ($K_D = 12$ nM), modeling the system as an arrangement of two binding motifs joined by a disordered linker could explain global affinity and the effectiveness of the AdE1A protein in E2F displacement. NMR experiments confirmed AdE1A binds Rb through the residues in the E2F-like and LxCxE motifs, which are modulated by flanking residues with complementary function in the interaction and also through residues in the linker (TAZ2 binding region), indicating this linker acts as an entropic chain but also establishes a new set of interactions with Rb.

P1.27 Crystal structures of the activated response regulator RcsB reveal conformational dynamism for DNA interaction

PATRICIA CASINO.[§]

Universitat de Valencia, Valencia, Spain.

§patricia.casino@uv.es

The response regulator RcsB is the master effector of the Rcs phosphorelay system. This system is found in *Enterobacteriaceae* and is involved in motility, capsule production and cell division, features related with bacterial pathogenesis. The Rcs phosphorelay system is comprised basically by three proteins, a hybrid histidine kinase named RcsC, a phosphotransferase RcsD and RcsB. Autophosphorylation of RcsC, upon signal detection, triggers a phosphorelay cascade through the individual components of the system that results in RcsB phosphorylation. Phosphorylated RcsB dimerizes and acts as a transcriptional factor activating or inhibiting the appropriate genes for bacterial regulation. Interestingly, RcsB can act as a transcriptional factor to regulate gene transcription of additional genes by heterodimerizing with auxiliary transcriptional factors independently of phosphorylation. Gene regulation dependent and independent of phosphorylation suggests that RcsB could exploit different surface areas for dimerization and advances alternative conformations to regulate gene transcription. We have conducted structural and functional studies on RcsB that reveal the conformation competent to bind bona fide promoters for capsule production and motility as well as another conformation that confirms the dynamism of RcsB, which could explain alternative ways of gene regulation.

P1.28 Mechano-chemical characterization of membrane fission by dynamins

REBECA BOCANEGRA-ROJO,^{1,§} Sara de Lorenzo,¹ Ariana Velasco,² Vadim Frolov,² José López-Carrascosa,³ Borja Ibarra.¹

¹IMDEA Nanociencia, Madrid, Spain; ²Ikerbasque, Spain; ³Centro Nacional de Biotecnología, Madrid, Spain.

§rebeca.bocanegra@imdea.org

The dynamin family proteins are GTPases involved in multiple cellular functions involving membrane fission and fusion events. Dynamin is necessary for internalizing essential nutrients, is tightly coupled to cell signalling events, and has been linked to neuropathies and myopathies. The better characterized function of dynamin is the membrane fission in the clathrin dependent endocytosis pathway; as a vesicle invaginates, dynamin is recruited to its neck, where it oligomerizes as a spiral generating a dynamin-lipid tube that constricts upon GTP hydrolysis, causing the fission of the neck and release of the vesicle. The ability of dynamin to constrict mechanically the underlying lipid bilayer makes it unique among GTPases as a mechano-chemical enzyme. However, the mechano-chemical processes governing their operation at the molecular level are still under debate. Using a combined fluorescence and optical tweezers approach, we have developed an experimental assay to measure the real-time activities of Dynamin 1 and Dynamin 2 proteins as they constrict individual membrane nanotubes and the effect of GTP analogues on their reactions. Our results shed light into the mechanical operation of these enzymes and reveal significant differences between them.

P1.29 Deciphering the molecular mechanisms of imp dehydrogenase

RUBÉN MARTÍNEZ-BUEY,[§] David Fernández-Justel, José Luis Revuelta.

Metabolic Engineering Group, Universidad de Salamanca, Salamanca, Spain.

§ruben.martinez@usal.es

IMP dehydrogenase (IMPDH) catalyzes the rate-limiting step in the guanine nucleotide biosynthesis and plays crucial roles in cell proliferation. Despite IMPDH is the target of drugs with antiviral, immunosuppressive and antitumor activities, its physiological mechanisms of regulation remain largely unknown. Using the enzyme from *Ashbya gossypii*, we demonstrated that the binding of adenine and guanine nucleotides to the canonical nucleotide binding sites of the regulatory Bateman domain induces different enzyme conformations with significantly distinct catalytic activities. Thereby, we described a nucleotide-controlled conformational switch that allosterically modulates the activity of eukaryotic IMPDHs. The physiological relevance of this regulatory mechanism is stressed by the fact that missense mutations in IMPDH, associated to severe retinopathies, such as Retinitis Pigmentosa, map into the nucleotide binding sites and disrupt allosteric regulation. Additionally, human IMPDH possess the ability to self-assemble into mesoscale assemblies, such as purinosomes and cytoophidia, that are suggested to modulate the enzyme activity and play important roles in regulating cell metabolism by controlling intracellular nucleotide homeostasis. We discuss here the linkage between the nucleotide-controlled conformational switch, the allosteric regulation of the catalytic activity and the dynamics of cytoophidia in eukaryotic IMPDHs.

P1.30 Functional optimization of broadly neutralizing HIV-1 antibody 10E8 by promoting membrane interactions.

SARA INSAUSTI,^{1,§,§} Edurne Rujas,¹ Daniel P. Leaman,² Lei Ortigosa-Pascual,¹ Lei Zhang,² Michael B. Zwick,² José L. Nieva.¹

¹*Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country (UPV/EHU), Bilbao, Spain;* ²*Department of Immunology and Microbial Science, The Scripps Research Institute, La Jolla, California, USA.*

§sara.insausti@ehu.eus, §*Biophysical Society / IRC bursary*

The 10E8 antibody targets a helical epitope in the membrane-proximal external region (MPER) and transmembrane domain (TMD) of the envelope glycoprotein (Env) subunit gp41, and is among the broadest known neutralizing antibodies against HIV-1. Accordingly, this antibody and its mechanism of action valuably inform the design of effective vaccines and immunotherapies. 10E8 exhibits unusual adaptations to attain specific, high-affinity binding to the MPER at the viral membrane interface. Here, we hypothesized that by increasing the net positive charge in its basic paratope surface, the neutralization potency of the antibody may be enhanced. We found that increased positive charge at this surface strengthened an electrostatic interaction between antibody and lipid bilayers, enabling 10E8 to interact spontaneously with membranes. Notably, the modified 10E8 did not gain any observable polyreactivity and neutralized with significantly greater potency. Binding analyses indicated that the optimized 10E8 bound with higher affinity to the epitope peptide anchored in lipid bilayers, and to Env spikes on virions. Overall, our data provide a proof-of-principle for rational optimization of 10E8 via manipulation of its interaction with the membrane element of its epitope, and emphasize the crucial role played by the viral membrane in the antigenicity of the MPER-TMD of HIV-1.

P1.31 Biophysical characterization of W196 mutants of the human apoptosis inducing factor

SILVIA ROMERO-TAMAYO,^{§,§} Raquel Villanueva, Milagros Medina, Patricia Ferreira-Neila.

Department of Biochemistry and Molecular and Cellular Biology and Institute for Biocomputation and Physics of Complex Systems, University of Zaragoza, Zaragoza, Spain.

§silvia4biotech@gmail.com, §*Biophysical Society / IRC bursary*

The Apoptosis Inducing Factor (AIF) was first described as a flavoprotein causing caspase-independent death when translocated to the nucleus. However, in healthy mitochondria, AIF contributes to the maintenance and stability of respiratory complexes being present in a monomer-dimer equilibrium modulated by NADH/NAD⁺ levels. AIF folds in three domains: FAD- and NADH-binding domains responsible for its NADH oxidoreductase activity, and C-terminal domain considered the pro-apoptotic region. When NADH reduced, hAIF_{D1-101} stabilizes a long-lived charge-transfer complex (CTC) whose structure revealed two NAD(H⁺) binding sites and the flavin in the reduced state. One of the coenzyme molecules binds to the NADH-binding domain and is implicated in the NADH reductase activity. The second molecule binds to the C-terminal in a cavity formed upon protein reduction. Noticeably, mutations of residues contributing to its binding are associated to several human neurodegenerative disorders. W196 is situated in a β -hairpin that connects the NADH- and FAD-domains to the C-terminal domain and suffers a considerably reorganization in its position upon flavin reduction to stabilize the second NADH molecule. We present the biophysical characterization of several site-directed mutants at W196 to investigate its contribution to hAIF stability, CTC stability, NADH binding and reductase activity.

P1.32 Functional analysis and crystallization of chloroplast E3 ubiquitin ligases

WOJCIECH BIALEK,[§] Natalia Lazarewicz, Ireneusz Gorniak.

University of Wrocław, Wrocław, Poland.

[§]wojciech.bialek@uwr.edu.pl

The presence of different E3 ligases have been confirmed in many eukaryotic organelles, but only recently SP1, a RING-type ubiquitin E3 ligase, has been discovered in the chloroplast outer membrane of a model plant *Arabidopsis thaliana* (Ling and Jarvis, 2012). This membrane protein may decide upon the plastid's proteome, developmental fate, and functions by controlling the protein import in response to abiotic stresses by UPS (ubiquitin-proteasome system) (Ling and Jarvis, 2015). In addition, two homologs of SP1, SPL1 and SPL2, have also been identified but their roles remain obscure. To confirm their putative enzymatic activities we heterologously expressed full-length AtSP1 and its homologs AtSPL1 and AtSPL2 in *E. coli* and performed both *in vitro* and *in vivo* ubiquitination assays. We also confirmed their localization to chloroplast envelope by protoplast transformation and confocal microscopy. As a pre-crystallization screening of membrane proteins, we employed fluorescence-detection size-exclusion chromatography-based thermostability assay (FSEC-TS) (Hattori *et al.*, 2006) to assess the effects of different detergents and lipids on protein stability. Here we show initial crystallization results using commercially available crystallization screens.

P1.33 Measurements of the hydrodynamic protein size for the quantitative analysis of binding induced conformational change

HANNA MUELLER-LANDAU,[§] Friederike Moeller, Wolfgang Kaiser, Ulrich Rant.

Dynamic Biosensors GmbH, Martinsried/Planegg, Germany.

[§]mueller-landau@dynamic-biosensors.com

Molecular interactions and protein function crucially depend on protein structure and often involve conformational changes. switchSENSE provides a unique platform for the analysis of conformational changes in proteins by combining high sensitivity kinetics with structural analysis. Here, we present results for the analysis of conformational changes and allosteric regulation in small proteins (kinases, STING) as well as large proteins (transglutaminase, insulin receptor). In addition to absolute Stokes radii and conformational changes, experiments can yield KD values and kinetics (on- and off-rates). The proteins are immobilized on gold microelectrodes on a biochip via electro-switchable DNA nanolevers using generic conjugation methods. The DNA nanolevers are actuated by alternating electric fields and are used to sway the proteins through the solution close to the surface at high-frequencies. The speed of this oscillatory movement is observed in real-time by fluorescence energy transfer. As the speed of the protein motion depends on its hydrodynamic drag, it can be directly converted to the protein's Stokes diameter. Real-time monitoring of the switching speed allows to resolve binding kinetics and to detect structural effects, such as conformational changes or multi-/monomerizations and thus provides quantitative and easy-to-interpret means for the analysis of protein size and shape.

P2 | Membrane Structure and Function

Hall Posters and Exhibitors Hall

Odd-numbered posters

Date: Thursday, June 21st

Time: 19:00-20:00

Even-numbered posters

Date: Friday, June 22nd

Time: 12:00-13:00

P2.1 Anthracyclines interaction with lipid model membranes: Implications for cell type selectivity

CLÁUDIA NUNES,^{1,§} Ana Catarina Alves,¹ Miguel Horta,¹ Aniket Magarkar,² Alex Bunker,³ Salette Reis.¹

¹LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Porto, Portugal; ²Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic, Prague, Czech Republic; ³Faculty of Pharmacy, University of Helsinki, Helsinki, Finland.

§cdnunes@ff.up.pt

The anthracyclines drug-membrane interactions play a crucial role in the molecules bioavailability and drug-induced toxicity processes. In fact, the cell boundary is involved in several biological activities that lead to cell survival, differentiation and death. Thus, structural changes in the lipid phase induced by these drugs can disturb the membrane function and indirectly modulate membrane proteins activity. Additionally, cancer cells membranes present diverse chemical, structural, metabolic and biophysical characteristics that are different from normal cells. In this context, 2d and 3d lipid membrane mimetic models of cancer and normal cells were designed and conjugated with multiple biophysical techniques, to assess the anthracyclines location and impact on the structure and dynamics of lipid membranes. The results show that these interactions are intrinsically dependent on the lipid composition, the membrane physical state and structure. Moreover, the small structural difference between daunorubicin and doxorubicin (which presents a hydroxyl instead of a methyl group in the side chain) has a significant impact in the interactions and even conditions the target cancer for each one is used.

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P2.2 Emulating the effect of breathing dynamics on pulmonary surfactant interfacial drug-delivery capabilities by a novel biophysical model

CRISTINA GARCÍA-MOUTON,^{1,2,§,§} Alberto Hidalgo,^{1,2} Francesca Salis,³ Guillermo Orellana,³ Jesús Pérez-Gil,^{1,2} Antonio Cruz.^{1,2}

¹Departamento de Bioquímica y Biología Molecular, Facultad de Biología, Universidad Complutense de Madrid, Madrid, Spain; ²Instituto de Investigación Sanitaria Hospital 12 de Octubre (imas12), Madrid, Spain; ³Departamento de Química Orgánica, Facultad de Química, Universidad Complutense de Madrid, Madrid, Spain.

§crigar12@ucm.es, §[Biophysical Society / IRC bursary](#)

Pulmonary surfactant (PS) is a lipid-protein complex synthesized, assembled and secreted by type II pneumocytes, and is essential to reduce the surface tension at alveolar surface during exhalation. The particular composition and structure of surfactant complexes allow their rapid adsorption and diffusion along the air-liquid interface. These properties are particularly well suited to facilitate the solubilization and delivery of different types of drugs and nanoparticles in combination with PS into the lungs. PS capabilities to act as a drug delivery system have been already demonstrated, and the aim of this work has been to evaluate the effect of breathing-like compression-expansion interfacial dynamics on this efficiency. A novel setup developed in our laboratory, consisting of a double through combining Langmuir-Blodgett and Wilhelmy balances, has been used to emulate the respiratory interface during compression-expansion cycles. Combined pressure-area-time isotherms obtained using this device helps to predict the behaviour of different PS/drug and PS/nanoparticle combinations and how the different cargos might be released from the alveolar interface upon compression-expansion cycles. According to these results, we propose a model of how respiratory dynamics could enhance PS capabilities in terms of drug vehiculization to the distal airways and the subsequent drug release once at the alveolar spaces.

P2.3 Biophysical interactions between omeprazole and lipid membrane models: Unveiling an additional mechanism of action

DANIELA PRISCILA RODRIGUES-LOPES-DE-CAMPOS,^{1,§,§} Cláudia Nunes,¹ Catarina Pereira-Leite,¹ Philippe Fontaine,² Bruno Sarmento,³ Sven Jakobtorweihen,⁴ Salette Reis.¹

¹LAQV, REQUIMTE, Faculdade de Farmácia, Universidade do Porto, Portugal; ²Synchrotron SOLEIL, L'Orme des Merisiers, Saint Aubin, Gif-sur-Yvette, France; ³INEB - Instituto de Engenharia Biomédica and i3S - Instituto de Investigação e Inovação em Saúde, Universidade do Porto, and IINFACTS, Instituto de Investigação, Porto, Portugal; ⁴Technische Universität Hamburg, Hamburg, Germany.

§dplopes@ff.up.pt, §EBSA bursary

In vivo studies showed that omeprazole has a cytoprotective effect on the gastric mucosa that is not related with its ability to inhibit the proton-pump (1). moreover, this therapeutic effect was not verified when omeprazole was given intravenously. this raises the hypothesis that the ph may be the key to understand the mechanism behind this effect, which is not fully understood yet. hence, we studied the impact of the protonation states of omeprazole on the topical action on dipalmitoylphosphatidylcholine membrane models. different biophysical techniques were used, namely, langmuir monolayers pressure-area isotherms, brewster angle microscopy, infrared reflection-absorption spectroscopy measurements, x-ray diffraction studies, including small-angle, wide-angle, and grazing incident x-ray diffraction, and molecular dynamics simulations. the results showed that the protonated state of omeprazole is able to intercalate between dppc molecules, promoting the formation of domains with highly condensed and untilted phospholipids. the interaction is driven by both electrostatic and hydrophobic interactions. hence, the ability of omeprazole in its charged state to act as a phospholipid-like drug can be an additional mechanism to protect the gastric mucosa.

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P2.4 Specific surface functionalization of liposome with bioactive peptides

DIEGO DE LA FUENTE-HERRERUELA,^{1,§} Aarón Morallón-Piña,¹ Fabrice Giusti,² Paolo Natale,¹ Iván López-Montero.¹

¹*Dto. Química Física I, Universidad Complutense de Madrid, Madrid, Spain and Instituto de Investigación Hospital Doce de Octubre, Madrid, Spain;* ²*Institut de Biologie Physico-Chimique. CNRS, Paris, France.*

§dfuente@ucm.es

The design of efficient drug delivery vectors requires versatile formulations able to simultaneously direct a variety of molecular targets and to successfully escape the endocytic recycling pathway of host cells. Liposomal-based vectors need to be decorated on their lipid surface with specific peptides to fulfill the functional requirements of liposome absorbance, targeting and eventually content delivery. In general, the unspecific binding of functional peptides to the lipid surface often results in uncontrolled formulations that lose their surface functionality that hampers a successful drug delivery. In this work we present a straightforward synthesis strategy to anchor cysteine containing functional peptides to thiol-reactive lipids for controlled liposomal formulations. This proof-of-concept focuses on the conjugation of the truncated Fibroblast Growth Factor (FGF), needed for cell targeting, and the conjugation of the GALA peptide, a pH sensitive and fusogenic peptide for endosomal escape, to a lipid moiety that was further used for the formulation of surface active liposomes. Optimization of the amount of FGF and GALA at the lipid surface leads to an improved liposome uptake in mouse embryonic fibroblasts and a significant endosomal escape that was visualized with a fluorescent tracer that was incorporated into the lumen of the liposomes.

P2.5 Lamellar phases of n-palmitoyl and egg sphingomyelin: A comparative study.

EMILIO JOSÉ GONZÁLEZ-RAMÍREZ,^{1,§,§} Zoran Arsov,² Alicia Alonso,¹ Félix M. Goñi,¹ Stephanie Tristram-Nagle,³ John F Nagle.³

¹*Instituto Biofisika (CSIC, UPV/EHU), and Departamento de Bioquímica, Universidad del País Vasco (UPV/EHU), Bilbao, Spain;* ²*Department of Condensed Matter Physics, Laboratory of Biophysics, Jozef Stefan Institute, Ljubljana, Slovenia;* ³*Biological Physics Group, Physics Department, Carnegie Mellon University, Pittsburgh, Pennsylvania, USA.*

§gr.emiliojose@gmail.com, §*Biophysical Society / IRC bursary*

Recent interest in SMs arises from their interaction with cholesterol in generating cholesterol-rich lateral membrane domains, their specific binding to and regulation of particular membrane proteins, and their involvement as precursors to simpler sphingolipids in cell signaling events. Egg SM (ESM) stands out as the most homogeneous of the natural SMs. Even the nature of the generic SM main transition has been unclear, by analogy with saturated PC lipids; the transition is often inferred to be from ripple phase to fluid (liquid-crystalline or liquid-disordered) phase if a pretransition is observed and gel-to-fluid phase if it is not. This work studies both PSM and ESM in the temperature regime from 3°C to 55°C using X-ray diffraction and X-ray diffuse scattering on hydrated, oriented thick bilayer stacks. The aim of this work is to characterize the structure of the phases, particularly for $T < T_M$, using oriented hydrated samples. We observe clear evidence for a ripple phase for ESM in a large temperature range. This unusual stability of the ripple phase was not observed for PSM. Our study demonstrates that oriented lipid films are particularly well-suited to characterize ripple phases since the scattering pattern is much better resolved than in unoriented samples.

P2.6 Molecular dynamics of small molecules adsorbed at zwitterionic membranes

HUIXIA LU,[§] Jordi Martí.

Technical University of Catalonia, Barcelona, Spain.

[§]huixia.lu@upc.edu

From full-atom Molecular Dynamics simulations of aqueous lipid bilayer zwitterionic membranes, formed by di-palmytoil-phosphatidyl-choline phospholipids, cholesterol and one small molecule (one tryptophan or one melatonin molecule) and solvated by flexible TIP3P water molecules and sodium chloride ions at human body concentration and temperature, we computed microscopic structure and dynamics of the system. The simulation experiments were performed using the NAMD-2.7 package, operating by means of the CHARMM36¹ force field. Radial distribution functions, potentials of mean force, spectral densities and diffusion coefficients have been computed and are reported in the poster. After a reliability test was reported on tryptophan², individual contributions of all components of the molecular probes have been computed separately and related with global properties of the membrane. Self-diffusion of the small molecules reveals a variety of time scales playing a role in membrane dynamics and their spectral densities are in good agreement with experimental data. A competition between the effects of cholesterol and of the small molecules on the shape and structure of the membrane has been observed.

P2.7 Characterization of model membranes by dsc and drug partition by ITC

INÊS OLIVEIRA-MARTINS,^{1,§} Maria J. Moreno,² Margarida Bastos.¹

¹CIQ-UP Dep. Chemistry and Biochemistry, FCUP, Porto, Portugal; ²Biological Chemistry Group, Chemistry Department FCTUC, Coimbra, Portugal.

[§]up201100312@fc.up.pt

The failure to cross the blood-brain barrier (bbb) is a limiting factor in the development of drugs for diseases that affect the brain (e.g. dementias) affecting millions of people worldwide. many drugs are not recognized by active transporters and thus passive permeation through the membrane is a significant route¹. Model membranes with appropriate composition provide a refined model to assess passive permeation. in this work we used two calorimetric methods to characterize the membranes' phase behavior (dsc)² and the partition of a model drug, chlorpromazine, to these membranes (ITC)³. Binary mixtures of DMPC, DMPE and POPS were used. The obtained thermodynamic parameters for the partition of chlorpromazine obtained now are compared with the ones previously obtained for model membranes of a different composition⁴, to assess the importance of lipid and charge on drug partition.

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P2.8 Chasing the polarity of POPC/Chol bilayer membranes with pyrene fluorescence

JOANA CRISTO,^{1,§} Jorge Martins.²

¹CBMR - Universidade do Algarve, Algarve, Portugal; ²CCMAR and CBMR, Universidade do Algarve, Algarve, Portugal.

§irishipo@gmail.com

To study the polarity of lipid bilayers one can use fluorescent probes as pyrene (Py), since this polycyclic aromatic hydrocarbon displays a variation in emission spectra which is related with the polarity of its environment. The physical-chemical properties of Py settle its location in the ordered section of the methylenic palisade of lipid bilayers, stating their values of dielectric constants, averaged transversally in space (the longest axis of Py, 9.2 Å) and laterally in time (due to lateral diffusion of Py during its fluorescence lifetime, ~150 ns in aerated media). Py has a well-defined emission spectrum composed by 5 bands, and polarity can be estimated by calculating the ratio of the fluorescence intensities of the first band (I_1) and the third (I_3). From correct measurements of the ratio I_1/I_3 in lipid bilayers (without spectral distortions), one can estimate their dielectric constant, provided that a calibration plot is constructed with standard alcoholic solvents. The bilayer polarity of 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phos-phocholine (POPC) and its binary mixtures with cholesterol were monitored using the Py Ham Effect (I_1/I_3). Pure POPC exhibits higher dielectric constant than the mixtures at high cholesterol (Chol) proportions, pointing to features observed in the available thermal phase diagrams.

P2.9 Effect of a synthetic antitumoral catechin on a anionic phospholipid membrane as studied by FT-IR spectroscopy and molecular dynamics simulation

JOSÉ ANTONIO TERUEL-PUCHE,[§] Antonio Ortiz-López, Francisco José Aranda-Martínez.

Departamento de Bioquímica y Biología Molecular A, Facultad de Veterinaria, Universidad de Murcia, Murcia, Spain.

§teruel@um.es

A synthetic derivative of a green tea polyphenol, 2-O-(3,4,5-trimethoxybenzoyl)-(-)-catechin-3-gallate (TMCG) has showed antiproliferative activity against malignant melanoma. The hydrophobic nature of TMCG suggests that the interaction with membranes would be important to understand the underlying molecular mechanism, and its potential capacity to modulate membrane related processes. We look into the interactions of TMCG with model systems composed of anionic phospholipids membranes (dimiristoylphosphatidylserine, DMPS) by using infrared spectroscopy in the carbonyl stretching vibration spectral region. To extract qualitative information about changes in phospholipid hydration, changes in the absorption maximum of the carbonyl stretching band were measured. It was observed an important increase in the hydration of the interfacial region of the bilayer in the fluid phase in the presence of TMCG. Molecular dynamics approach has been shown to be a helpful tool to examine the dynamics of atomic-level phenomena and hence molecular interactions. In this work, molecular dynamics simulations were carried out to characterize the molecular structure and dynamics of DMPS-TMCG bilayers. Different properties were calculated to interpret the experimental results at molecular level, such as the number of hydrogen bonds in the lipid and TMCG molecules, area per lipid, TMCG localization in the bilayer and inter molecular interactions.

P2.10 Comparing the structure and surface activity of pulmonary surfactant samples purified from bronchoalveolar lavage of porcine lung and human amniotic fluid

JOSÉ CARLOS CASTILLO-SÁNCHEZ,^{1,§,§} Emma Batllori-Badia,² Alberto Galindo,³ Jesús Pérez-Gil,¹ Antonio Cruz.¹

¹Biochemistry and Molecular Biology Department, Faculty of Biology, Complutense University, Madrid, Spain and Research Institute Hospital Universitario "12 de Octubre" (imas12) and Complutense University, Madrid, Spain; ²Department of Obstetrics and Gynaecology. Hospital Universitario 12 de Octubre, Madrid, Spain; ³Research Institute Hospital Universitario "12 de Octubre" (imas12) and Complutense University, Madrid, Spain and Department of Obstetrics and Gynaecology. Hospital Universitario 12 de Octubre, Madrid, Spain.

§josecarc@ucm.es, §SBE bursary

Pulmonary surfactant (PS) is an essential lipoprotein complex that reduces surface tension to stabilize the alveolar air-liquid interface. PS is synthesized and stored by alveolar type-II cells into highly packed organelles called lamellar bodies (LBs). It has been recently proposed that newly produced PS assembled into LBs exhibits a particularly active state, which is partially lost along the respiratory compression-expansion cycles. PS is commonly purified from bronchoalveolar lavages of animal lungs and, after that, lipids and surfactant hydrophobic proteins are extracted using organic solvents and reconstituted as buffered suspensions to be used as standard surfactant samples. The properties of newly synthesized PS are compromised by this methodology and standard surfactants scarcely contain true freshly secreted LBs. A new approach has been proposed to purify relatively high amounts of PS preserving the properties of freshly secreted LBs from human amniotic fluid. Here, we compare the molecular structure and surface-active properties of PS membranes purified from BAL of porcine lungs and those purified from AFS. We have observed meaningful structural differences in the organization of lipid regions in interfacial films from these two materials, which may be related to clear differences in their behaviour at the air-liquid interface.

P2.11 How is the interaction of the C1B domain of PKC ϵ with membrane phospholipids?

JUAN CARMELO GÓMEZ-FERNÁNDEZ,[§] C. Juan, Antonio L. Egea-Jiménez.

Universidad de Murcia, Murcia, Spain.

§jcgomez@um.es

C1 domains are members of the Cys-rich domains superfamily, formed by 50-51 amino acids residues present in many types of proteins, as it is the case of the classical and novel Protein Kinases C (PKCs). Both types of PKCs, classical and novel isoenzymes, possess two C1 subdomains, C1A and C1B, although it is not totally clear why two modules are needed. C1 domains are known to interact with diacylglycerol and exogenous agents like phorbol esters. In this work we have studied the role of positively charged amino acids residues located on top of the C1B ϵ domain and their involvement in the interaction membrane-protein. More specifically, K251, R268, R282 and R283 were replaced by alanine and we characterized the effect of single, double and triple mutations. Results show that binding is decreased by increasing of the number of residues mutated in the domain, and it be even abolished in the presence of diacylglycerol. In conclusion, the electrostatic interactions derived of these positively amino acids residues is important to give place to membrane docking which is further stabilized by interaction with the diacylglycerol.

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P2.12 Minimal requirements for mitochondrial division: Lessons from an in vitro system

JUAN MANUEL MARTÍNEZ-GÁLVEZ,^{§,§} Anna V. Shnyrova.

Biofisika Institute (CSIC, UPV/EHU) and Department of Biochemistry and Molecular Biology, University of the Basque Country, Leioa, Spain.

[§]juanmanuel.martinez@ehu.eus, [§] *SBE bursary*

Mitochondria, the double membrane-bound organelles, are best known as the powerhouse of eukaryotic cells. In the last decades, the rich dynamical behavior of mitochondrial networks, namely mitochondrial splitting apart and their merging together, has drawn much attention due to its potential implications in many pathological processes and aging. In particular, mitochondrial splitting or division (MD) enables maintenance of a healthy mitochondrial reticulum, and its misbalance leads to cell pathologies through still poorly understood mechanisms. MD requires fine kinetic control over topological transformations of both the inner and outer mitochondrial membranes, achieved largely through synergistic action of proteins from the dynamin (DNM) superfamily. Recently, we have developed a lipid nanotube based membrane system mimicking the topology of mitochondria. We used this system to characterize the minimum force requirements for the MD process and to see how DNMs catalyse the membrane rearrangements during MD. We hope to further use this in vitro tool to test different protein/lipid factors involved in the MD in cells, thus establishing the molecular details of the MD process and its alterations leading to pathologies important at the cellular level.

P2.13 Fullerene interaction with lipid membranes

JUDIT SASTRE-POZO,[§] Víctor Almendro-Vedia, Iván López-Montero.

Fundación Investigación Biomédica Hospital 12 de Octubre (I+12), Madrid, Spain. Departamento Química Física, Facultad de Ciencias Químicas, UCM, Madrid, Spain.

[§]judit_sastre@hotmail.com

Fullerenes are a series of hollow carbon molecules that form either closed cages or cylinders. among the multiple carbon fullerenes shapes, the most form abundant is carbon 60 (c60). the cytotoxic effects described for fullerenes prevent their many potential biological uses¹ since they are poorly understood. Fullerenes are poorly soluble in most polar solvents² and interacts with hydrophobic moieties such as those provided by lipid bilayers³. A better understanding of fullerene interaction with lipid membranes is needed if these materials are to become biologically useful. In this work, model lipid membranes build as lipid vesicles and supported lipid bilayers of different compositions are used to investigate fullerene C60-lipid interaction. By means of fluorescence-based techniques we show that C60 fullerenes decrease both the spontaneous flip-flop rate and the diffusion coefficient of lipid bilayers. In addition, preliminary results show that C60 fullerenes promotes phase interleaflet domain alignment in lipid bilayers membranes displaying Lo/Ld phase separation.

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P2.14 Impact of Ca^{2+} -dependent PI(4,5)P2 clustering on the properties of PI(4,5)P2 binding proteins

LUÍS PEDRO BORGES-ARAÚJO,^{1,§,§} M. E. Monteiro,¹ D. Mil-Homens,² N. Bernardes,² M. J. Sarmiento,¹ A. Coutinho,¹ M. Prieto,¹ F. Fernandes.¹

¹Centro de Química-Física Molecular and IN-Instituto de Nanociência e Nanotecnologia, Instituto Superior Técnico, Universidade de Lisboa, Lisbon, Portugal.; ²iBB-Institute for Bioengineering and Biosciences, Biological Sciences Research Group, Lisbon, Portugal.

§lpborgesaraujo@tecnico.ulisboa.pt, §EBSA bursary

Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P2) is a key modulator of eukaryotic plasma membrane associated signalling events. Studies on the impact of physiological divalent cation concentrations on PI(4,5)P2 clustering, suggest that protein anchoring to the membrane through PI(4,5)P2, is not defined solely by a simple (monomeric PI(4,5)P2)/(protein bound PI(4,5)P2) equilibrium, but instead involve interactions with PI(4,5)P2 clusters. Nevertheless, the impact of the complex PI(4,5)P2 organization on its interactions with binding proteins is largely unknown. Using advanced spectroscopic methodologies (FRET, FCS and PCH), we characterized the impact of calcium on the dynamics of pleckstrin homology (PH) domains tagged with a fluorescent protein. We show that in Giant Unilamellar Vesicles (GUVs) presenting PI(4,5)P2, the membrane diffusion properties of PH-FP are affected by the presence of Ca^{2+} , suggesting interaction of the protein with PI(4,5)P2 clusters. Importantly, PH-FP is found to dimerize in the membrane in the absence of Ca^{2+} and this oligomerization is inhibited in the presence of physiological concentrations of the cation. Furthermore, Ca^{2+} induced clustering of PI(4,5)P2 enhanced protein sequestration of the phosphoinositide, depleting the levels of free PI(4,5)P2. These results confirm that Ca^{2+} -dependent PI(4,5)P2 clustering has the potential to influence affinity, oligomerization and organization of PI(4,5)P2 binding proteins in the plasma membrane.

P2.15 Cardiolipin effects on membrane structure: From bacteria to mitochondria.

SÍLVIA LOPES,^{§,§} Paula Gameiro.

LAQV @Requimte, University of Porto, Porto, Portugal.

§silvia.lobes@fc.up.pt, §EBSA bursary

Cardiolipin (CL) plays a key role in dynamic organization of bacterial and mitochondrial membranes. CL is a minor component of the plasma membrane in many types of bacteria (5–30% depending on the bacteria and stage in the life cycle) and in the mitochondria (5–10% in the outer membrane and about 20% in the inner membrane). Is a lipid that contains four acyl chains and tends to form nonlamellar structures. These structures are believed to play a key role in membrane structure and function. CL alterations, in mitochondria, have been linked to disorders such as Barth syndrome and Parkinson's disease. However, the molecular effects of CL on membrane organization remain poorly understood. Choosing natural extracts of CL mixtures, of *E. coli* and bovine heart, several biophysical studies were conducted to study its impact on model membrane properties. Results showed that CL presence/absence in membrane models (of bacteria or mitochondria) dramatically changes membrane's properties such as thermotropic properties, fluidity and interaction with membrane active compounds. Due to this major impact, we believe that CL inclusion, which is simple from a practical standpoint, represents a major improvement in the construction of better model systems both for bacterial and mitochondrial membranes.

P2.16 Effect of psychosine on model lipid membranes

YENISLEIDY DE LAS MERCEDES ZULUETA-DÍAZ,^{1,§} Maria Laura Fanani,² Sofía Caby.¹

¹Universidad Nacional de Córdoba. Facultad de Ciencias Químicas. Departamento de Química Biológica "Ranwel Caputto". Córdoba, Argentina; ²CONICET, Córdoba, Argentina.

§yzulueta@fcq.unc.edu.ar

Psychosine or β -galactosylsphingosine (PSY) is a toxic intermediate in the biosynthesis of sphingolipids. Its accumulation into the cell has been proposed as responsible for the Krabbe disease. Several evidences propose that this amphiphilic molecule exerts its pathological effect by partitioning into membranes and affecting its function. In this work, we have studied how this cationic lipid affects electrostatic and rheological properties of the membranes. We explored the interaction of PSY with phospholipid membranes at neutral pH in different phase state, used as classical model systems. Additionally, we explored PSY interaction with a complex lipid mixture of phospholipids, sphingolipids and cholesterol that mimic the lipid composition of myelin. For this purpose, we combined monolayer techniques, Brewster Angle Microscopy and Fluorescence, and Zeta Potential. Our results show that PSY exhibit surface activity and high sensitivity to the phase state. Partitioning preferentially into expanded phases in phospholipid membranes. This result suggests that PSY may stabilize phase separation; affecting the subtle balance that regulates phase separation in myelin. Furthermore, we observed that PSY alters the surface electrostatic of lipid membranes in a complex manner. Being the Krabbe disease a demyelinating process, our results are relevant to the supramolecular interpretation of the mechanism of biomembrane perturbation by PSY.

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Date: Thursday, June 21st

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P3.1 Unveiling the TRPV2 binding site by combining drug screening experimental and computational methods

ALEX PERALVAREZ-MARÍN,^{1,§} Marco Martins,¹ Víctor A. Lórenz-Fonfría,² Christian Jorgensen,³ Antonio Alcaraz,⁴ Ute Hellmich,⁵ Yuji Furutani,⁶ Carmen Domene,⁷ Rachele Gaudet.⁸

¹Unitat de Biofísica, Universitat Autònoma de Barcelona, Facultat de Medicina, Cerdanyola del Vallés, Spain;

²Institute of Molecular Science, Universitat de València, Paterna, Spain; ³Kings College London, Department of Chemistry, London, United Kingdom; ⁴Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Castellón, Spain; ⁵Institute for Pharmacy and Biochemistry, University of Mainz, Germany; ⁶Department of Life and Coordination-Complex Molecular Science, Institute for Molecular Science, Okazaki, Japan; ⁷Chemistry Research Laboratory, University of Oxford, Oxford, United Kingdom / Department of Chemistry, University of Bath, Bath, United Kingdom; ⁸Department of Molecular and Cell Biology, Harvard University, Cambridge, MA, USA.

§alex.peralvarez@uab.cat

The transient receptor potential vanilloid 2 (TRPV2) ion channel is an ubiquitous membrane protein responding to several physical stimuli, such as heat, mechanical stretch, osmotic changes, etc. However, few chemical stimuli have been identified for TRPV2, and the few that have been identified are non-specific for this channel. In this study, we take advantage of a structural biology workflow in *Pichia pastoris* to obtain large quantities of recombinant TRPV2 and setup a yeast membrane-based drug screening protocol. This protocol allowed us to validate the specificity of several known drugs for TRPV2 and to identify new ones in a mid-throughput scale fashion. In addition, we modeled the binding to TRPV2 for some of the drugs tested in our yeast membrane-based protocol, using *in silico* computational-aided methods such as molecular dynamics simulations, docking, and free-energy calculations. This study opens new opportunities to improve the pharmacological toolbox for TRPV2, an almost drug-orphan somatosensory ion-channel.

P3.2 PKC-dependent Kv1.3 endocytosis

ANTONIO FELIPE,^{1,§} Katarzyna Styrzczewska,¹ Mireia Pérez-Verdaguer,¹ Albert Vallejo-Gracia,¹ Nuria Comes,¹ Alexander Sorkin,² Antonio Felipe.¹

¹Universitat de Barcelona, Barcelona, Spain; ²University of Pittsburgh, Pittsburg, USA.

§afelipe@ub.edu

The voltage-dependent potassium channel Kv1.3 participates in the immune system physiology. Kv1.3 regulates the membrane potential triggering downstream Ca²⁺-dependent pathways. This channel concentrated in specific membrane microdomains serving as signaling platforms. Altered expression of Kv1.3 is observed at the onset of several autoimmune diseases. We show that adenosine (ADO), acting as a potent endogenous modulator, stimulates PKC, thereby causing immunosuppression. PKC activation down-regulates Kv1.3 triggering a clathrin-mediated internalization that targets the channel to lysosomes. Thus, the amount of Kv1.3 at the plasma membrane decreases, which is clearly compatible with an effective anti-inflammatory response. This mechanism involves ubiquitination of Kv1.3, catalyzed by the E3 ubiquitin-ligase Nedd4-2. Postsynaptic density protein 95 (PSD-95), member of the MAGUK family, situates Kv1.3 into lipid-raft microdomains impairing the ubiquitination and endocytosis of the channel. Therefore, the association of PSD-95 with Kv1.3 would modulate the anti-inflammatory response in leukocytes. This work elucidates the PKC-dependent molecular mechanisms that target Kv1.3 during immunomodulation in leukocytes.

Financial support: Supported by MINECO and FEDER (BFU2014-54928-R and BFU2017-87104-R), Spain.

P3.3 Multiple binding sites of tetracaine in the muscle-type nicotinic acetylcholine receptor

Raúl Cobo,¹ Magdalena Nikolaeva,² ARMANDO ALBEROLA-DIE,^{1,§} Gregorio Fernández-Ballester,² Manuel González-Ros José,² Isabel Ivorra,¹ Andrés Morales.¹

¹División de Fisiología, Departamento de Fisiología, Genética y Microbiología, Universidad de Alicante, Alicante, Spain; ²Instituto de Biología Molecular y Celular, Universidad Miguel Hernández, Elche, Alicante, Spain.

§alberoladie.armando@ua.es

The mechanisms of action of many molecules, some of therapeutic relevance, on the nicotinic acetylcholine (ACh) receptor (nAChR) are poorly understood yet. We have previously shown that lidocaine acts on nAChRs by different means, because of its presence as charged and uncharged forms. Since tetracaine (Ttc) is almost exclusively present as a positively charged molecule at physiological pH, we have now explored its mechanisms of action on nAChRs. By combining electrophysiological experiments and *in silico* docking assays, using nAChR models in the closed and open states, we have found that Ttc binds to nAChRs at different sites, involving extracellular and transmembrane domains. Extracellular binding sites seem relevant for the closed-channel blockade whereas two sites within the pore, with different affinities for Ttc, contribute to open-channel blockade and enhancement of nAChR desensitization, respectively. Our results demonstrate a heterogeneity of Ttc actions on nAChRs, and contribute to the understanding of the complex modulation of muscle-type nAChRs by local anesthetics. Furthermore, a tentative assignment of the main nAChR residues involved in these modulating actions is raised.

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P3.4 Nicotinic acetylcholine receptor and cholesterol: Lipid rafts, membrane asymmetry, structural conformation and functionality.

CAMILA FABIANI,[§] Jeremías Corradi, Silvia Antollini.

Instituto de Investigaciones Bioquímicas de Bahía Blanca CONICET-UNS, Departamento de Biología, Bioquímica y Farmacia, Universidad Nacional del Sur, Bahía Blanca, Argentina.

[§]cfabiani@inibibb-conicet.gob.ar

The muscle nicotinic acetylcholine receptor (AChR) has an extracellular domain which contains neurotransmitter-binding sites and a transmembrane domain that forms the ion channel pore and exhibits extensive contacts with the surrounding lipids. A correct allosteric coupling between both domains is crucial for AChR function. The AChR is present in high-density clusters in the muscle cell membrane where it localizes mainly in lipid-ordered domains (Lo) enriched in cholesterol and sphingolipids. We studied the relationship between the AChR and cholesterol in *T. californica* AChR-rich membranes and in model membranes containing purified AChR. Depletion of cholesterol by methyl- β -cyclodextrin, enrichment of cholesterol or cholesterol-hemisuccinate (in this latter case, asymmetric membranes were obtained), and oxidation of cholesterol using cholesterol oxidase were correlated with: i) AChR structural conformation by crystal violet fluorescent probe, ii) AChR functionality by electrophysiology, iii) augmentation/diminution of Lo domains by GUVs formation and fluorescence microscopy, and iv) AChR location in these domains by FRET or by detergent treatment and SDS-PAGE. Altogether, we observed that a change in the amount, distribution or oxidation of cholesterol impacts not only in the size and location of Lo domains and in the AChR preference for them, but also in the AChR functionality and AChR structural conformation.

P3.5 AQP5-mediated hydrogen peroxide transport improves cell resistance to oxidative stress

CLAUDIA RODRIGUES,^{1,§,§} Andreia Filipa Mósca,¹ Duarte Lopes,² Per Amstrup Pedersen,³ Fernando Antunes,⁴ Graça Soveral.¹

¹Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal;

²Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; ³University of Copenhagen, Department of Biology, Copenhagen, Denmark; ⁴Centro de Química e Bioquímica e Departamento de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

[§]crodrigues@ff.ulisboa.pt, [§][EBSA bursary](#)

Aquaporins (AQPs) facilitate water and glycerol permeation through membranes and a few isoforms can also permeate H₂O₂ (*peroxiporins*). Increased H₂O₂ levels misbalance cell redox reactions and may induce tumorigenesis. Recently, we evaluated H₂O₂ permeability of mammalian AQPs individually expressed in yeast and reported the ability of the rat AQP5-transformed yeast strain to conduct H₂O₂. Sequence alignment of human and rat AQP5 isoforms show a sequence identity of 91%. Therefore, we next investigated H₂O₂ permeation by human AQP5 and related cell resistance to oxidative stress. The results indicate that, similarly to AQP3, and AQP8, human AQP5 also permeates H₂O₂ and importantly, improves cell resistance under oxidative stress. In addition, the effect of anti-oxidant compounds on hAQP5-dependent H₂O₂ accumulation and cell growth was examined. Curcumin and naringenin enhanced cell resistance in AQP5-transformed yeast cells and anti-proliferative properties known for these compounds were attenuated in yeast cells expressing human AQP5. These data suggest an important role of AQP5 in oxidative stress resistance and point to a novel mechanism explaining AQP5 involvement in cancer.

P3.6 TRPC6 channels regulate the plasma membrane location of orai channels in breast cancer cells.

ISAAC JARDIN-POLO,[§] Raquel Diez-Bello, José J. López, Carlos Cantonero, Ginés M. Salido, Juan A. Rosado.

Department of Physiology, University of Extremadura, Cáceres, Spain.

[§]ijp@unex.es

Store-operated calcium entry (SOCE), mediated by STIM, Orai and TRP proteins, is the most relevant pathway that controls intracellular Ca²⁺ homeostasis in non-excitabile cells [1]. Alteration in SOCE has been linked to several diseases including cancer [2]. Breast cancer cells express both Orai1 and Orai3 channels. However, their expression and function varies from ER⁺ (where Orai3 expression is enhanced) to triple negative cells (being Orai1 the predominant). Our results show that the TRPC6 channel, which is overexpressed in the ER⁺ MCF7 and triple negative MDA-MB-231 breast cancer cell lines, associates with both Orai1 and Orai3 in resting and upon intracellular Ca²⁺ store depletion. Furthermore, we have found that TRPC6 knockdown impairs Orai surface expression in the plasma membrane resulting in a dramatic inhibition of SOCE and proliferation in the breast cancer cell lines. Altogether, our results show a role of TRPC6 in the regulation of SOCE and proliferation in breast cancer cells.

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P3.7 Endosomal escape of plasmonic gold nanoparticles mediated by listeria monocytogenes listeriolysin O toxin LLO

ISMAEL PLAZA-GARCÍA-ABADILLO,^{1,2,§} Vanessa Manzaneda-González,¹ Matic Kisovec,³ Diego de la Fuente-Herruela,^{1,2} Gregor Anderluh,³ Andrés Guerrero-Martínez,¹ Paolo Natale,^{1,2} Montero Iván López.^{1,2}

¹Dpto. Química Física I, Univ. Complutense de Madrid, Spain; ²Instituto de Investigación Hospital "12 de Octubre", Madrid, Spain; ³Dep. Mol. Biology and Nanobiotechnology, National Institute of Chemistry, Ljubljana, Slovenia.

[§]ismplaza@ucm.es

The pathogen *listeria monocytogenes* Listeriolysin O toxin (LLO) is a pore forming protein of the cholesterol-dependent cytolysin (CDC) family. During host infection the pH-dependent pore formation of the LLO toxin destabilizes the phagolysosome and this allows *Listeria* to escape from the endosomal system and to survive inside the host cells. In this work, we exploit the pH-dependent pore formation of the LLO toxin for endosomal escape during drug delivery. LLO wild-type and mutants, with a stronger pH-dependence, have been heterologously produced and purified in *E. coli*. The LLO WT and LLO H311A mutant were tested for functionality *in vitro* and then conjugated to functionalized gold nanoparticles (Au-NP) to probe the endosomal escape. This “proof-of-concept” will be further developed for further applications in plasmonic photothermal therapy of cancer cells (PPTT), the Au-NP convert non-harmful light into thermal energy through the interaction of laser radiation with the longitudinal localized surface plasmon resonance of the Au-NP. the resulting intracellular Au-NP heating has demonstrated great potential for killing cancer cells by induced apoptosis or photothermolysis.

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P3.8 Verapamil inhibits K2P potassium channels and induces a depolarization in cultured sympathetic neurons.

Salvador Herrera-Pérez, Lola Rueda-Ruzafa, María Silveira-Loureiro, Diego Fernández-Fernández, **J ANTONIO LAMAS-CASTRO**.[§]

University of Vigo (CINBIO), Vigo, Spain.

[§]antoniolamas@uvigo.es

Verapamil is a drug used in cardiac conditions such as angina pectoris or hypertension, exerting its therapeutic effect by blocking L-type Ca²⁺ channels expressed in the heart. In addition to this effect, verapamil is also known to modulate some potassium channels in cardiac cells. However, the effect of this drug on the Autonomic Nervous System, including two pore domain potassium channels (K2P), remains unknown. In this study we analyze the effects of verapamil on several potassium currents and on the resting membrane potential of Superior Cervical Ganglion (SCG) neurons. We used the Patch-clamp technique (Whole-cell) to show that verapamil slightly reduces transient A and delayed rectifier potassium currents and more strongly a riluzole-activated K2P current. Verapamil also depolarized the resting membrane potential but did not affect the firing of SCG neurons. Our results strongly suggest that verapamil may modulate the resting membrane potential through the inhibition of K2P channels.

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P3.9 Activation of potassium TREK channels by physiological temperature in nodose ganglion neurons

LOLA RUEDA-RUZAFA,[§] Salvador Herrera-Pérez, María Silveira-Loureiro, Diego Fernández-Fernández, José Antonio Lamas-Castro.

Biomedical Research Centre, University of Vigo, Vigo, Spain.

[§]lrueda@uvigo.es

The nodose ganglion (NG) is composed of A, Ah and C-type neurons which have an important role in regulating visceral afferent function. The aim of this study was to investigate whether native TREK channels were activated by physiological temperature like reported for heterologously expressed channels. To study TREK currents, perforated-patch whole-cell and cell-attached single-channel recordings were carried out in mouse nodose neurons in culture. Initially, neurons were recorded at room temperature (24 °C) and afterwards the temperature of the bath solution was increased to 37 °C. At -30 mV, increasing the temperature to 37 °C induced a rise in outward currents. Consistently, in current-clamp this increase in temperature also evoked a membrane hyperpolarization both at -30 and at -60 mV in the three cell types. Additional single-channel experiments confirmed that TREK channels show a low open probability at room temperature and this probability is strongly increased with temperature. Altogether, our results highlight an important role of TREK channels in setting neuronal excitability at physiological temperature levels and they could be important in temperature sensing mechanisms.

Financial support: Centro Singular de Galicia (accreditation 2016-2019). European Union (European Regional Development Fund -ERDF). MINECO: BFU2014-58999-P, BFU2015-70067-REDC. Xunta de Galicia: INB1-131H-2.

P3.10 The antiparasitic buphenium is a potent agonist of *Caenorhabditis elegans* levamisole-sensitive nicotinic receptors

ORNELLA TURANI,[§] Guillermina Hernando, Jeremías Corradi, Cecilia Bouzat.

Instituto de Investigaciones Bioquímicas de Bahía Blanca-CONICET-UNS, Bahía Blanca, Argentina.

[§]oturani@inibibb-conicet.gob.ar

Nicotinic acetylcholine receptors (nAChRs) are ligand-gated ion channels involved in neuromuscular transmission. In nematodes, muscle nAChRs are main targets of antiparasitic drugs. Nematode parasites contain three pharmacological classes of muscle nAChRs, which are activated by levamisole (L-type), nicotine (N-type) and buphenium (B-type). The free-living nematode *Caenorhabditis elegans* is a model of parasitic nematodes, useful for drug discovery. Because in *C. elegans* muscle only the N-AChR and L-AChR classes have been described, we explored the behavioral (by paralysis assays) and molecular actions (by patch clamp recordings) of the antiparasitic buphenium. As in parasites, buphenium produced spastic paralysis. A mutant strain lacking the L-AChR showed full resistance to buphenium, indicating that this receptor is the drug target. Buphenium activated L-AChRs from isolated larvae muscle cells, eliciting channel activity as that elicited by levamisole. The analysis revealed that it is a potent agonist of the L-AChR and an open-channel blocker at higher concentrations. In contrast, we demonstrated that it is a very low efficacious agonist of the mammalian muscle nAChR. Molecular docking studies proposed that buphenium can form key interactions required for activation in mammalian and nematode nAChRs, revealed differences with ACh binding, and provided explanations for the experimental results.

P3.11 Origin of desensitization in the light-gated ion channel channelrhodopsin

VÍCTOR A. LÓRENZ-FONFRÍA,^{1,§} Mattia Saita,² Franziska Pranga-Sellnau,² Tom Resler,² Ramona Schlesinger,² Joachim Heberle.²

¹*Universitat de Valencia, Valencia, Spain;* ²*Freie Universitaet Berlin, Berlin, Germany.*

[§]victor.lorenz@uv.es

Channelrhodopsins (ChRs) are light-gated cation channels containing an all-trans retinal as a chromophore. In spite their wide use to activate neurons with light, the photocurrents of ChRs rapidly decay in intensity under both continuous illumination and fast trains of light pulses, broadly referred to as desensitization. This undesirable phenomenon has been explained by two interconnected photocycles, each of them containing a non-conductive dark state (D1 and D2) and a conductive state (O1 and O2). While the D1 and O1 states correspond to the dark-state and P3(520) intermediate of the primary all-trans photocycle of ChR2, the molecular identity of D2 and O2 remains unclear. By performing comprehensive time-resolved UV/vis experiments on dark-adapted and pre-illuminated sample we show that the P4(480) state, the last intermediate of the all-trans photocycle, is photoactive. Its photocycle contains a red-shifted intermediate, I3(530), whose decay matches the decay of O2 to D2. This and other results indicate that the D2 and O2 states correspond to the P4(480) and I3(530) intermediates, connecting desensitization of ChR2 with the photochemical properties of the P4(480) intermediate. This finding has important practical consequences in strategies aiming to reduce the desensitization tendency of ChR2 for optogenetic applications.

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P4.1 AFM monitoring of lead(II)-induced cell death in red blood cells

Aritz B. García-Arribas, Hasna Ahyayauch, Félix María Goñi, ALICIA ALONSO. §

Instituto Biofisika (UPV/EHU, CSIC), and Departamento de Bioquímica, Universidad del País Vasco (UPV/EHU), Leioa, Spain.

§ alicia.alonso@ehu.eus

Atomic force microscopy (AFM) has been applied to the characterization of human red blood cells (rbc) at 23°C. erythrocytes were attached to ethanol-washed glass coverslips and fixed with glutaraldehyde to obtain AFM images, so that cell diameters and thicknesses could be measured. when Pb^{2+} was applied at 10 mM at 37°C for 10 minutes (all lead incubations were previous to fixation), erythrocytes underwent a morphological change due to the start of rbc apoptosis (eryptosis), losing their typical biconcave form to become planar, spiked, and, later, spherical (spherocytes). a progressive decrease in diameter was observed. interestingly, the use of clotrimazole, a suggested anti-apoptotic agent, inhibited Pb^{2+} effect. Furthermore, when clotrimazole-treated RBC were exposed to lead overnight, morphology was still preserved. As opposed to previous *in vivo* studies in mice exposed to Pb^{2+} [1] no additional reagents other than clotrimazole were needed to achieve inhibition of lead effect in human RBC. Lead-induced RBC death is considered to be ceramide-related [2]. This data is relevant in the context of sphingolipid signaling and cell death.

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P4.2 Studying the pathophysiology of chronic pain: Characterization of an *in vitro* pre-clinical nociceptor model

EVA MARÍA VILLALBA-RIQUELME,[§] Antonio Ferrer-Montiel.

Instituto de Biología Molecular y Celular. Universidad Miguel Hernández de Elche, Alicante, Spain.

[§]eva.m.villalba@gmail.com

At present, chronic pain has become one of the highest-impact diseases in society, affecting around 20% of the population. Cumulative evidences suggest that inflammation plays an important role in the generation and maintenance of chronic pain through the functional potentiation of the sensory neurons or nociceptors. However, little is known about the prolonged exposure of sensory neurons to these agents, as it occurs in chronic pain. To address this issue, we have established a primary culture of nociceptors that can survive up to 10 days *in vitro*. Using electrophysiological techniques, we determined the passive membrane properties, the action potential threshold and the percentage of tonic firing and spontaneous activity of the peptidergic population of neurons. The impact of long-term exposure (48h) of these nociceptor cultures to different inflammatory soups was investigated. After their exposure, the percentage of tonic and spontaneous firing was higher than in control conditions. These results suggest that inflammatory conditions increase the neuronal firing of peptidergic nociceptors, and that nociceptor sensitization remains after insult removal. Our long-term nociceptor culture constitutes a primordial pre-clinical tool for understanding the functional and molecular changes underlying the chronification of pain.

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P4.3 Efficient expression of OPA1 mitochondrial membrane fusion protein in *in vitro* and *in vivo* experiments by using gemini/dope-based lipoplexes

MÓNICA MUÑOZ-ÚBEDA,^{1,§} Martina Semenzato,² Anais Franco-Romero,² Elena Junquera,³ Emilio Aicart,³ Luca Scorrano,² Iván López-Montero.¹

¹*Instituto de Investigación Biomédica Hospital 12 de Octubre (i+12), Madrid, Spain;* ²*Fondazione per la Ricerca Biomédica Avanzata, Venetian Institute of Molecular Medicine (VIMM), Padova, Italy;* ³*Facultad C.C. Químicas, Dpto. Química Física I, Madrid, Spain.*

[§]monicamubeda@gmail.com

Recent advances on the mitochondrial morphology indicate that mitochondria are able to form a highly dynamic network that constantly fuse and divide. Fusion and fission balances are very important for normal cellular function. In mammalian cells, there are three main proteins involved in the mitochondrial fusion: Mfn1 and Mfn2 (OMM) and OPA1 (IMM). Deletion of any of them in mouse embryonic fibroblasts (MEFs) and CD-1 mouse models produces mitochondrial fragmentation, thus leading to mitochondrial diseases (MD), to which there is no cure yet. In this work, lipoplexes have been conceived as efficient therapeutic agents against MD. Lipoplexes, lipid/DNA highly packed complex, are composed by Imidazol Gemini/DOPE mixed cationic liposomes with a plasmid DNA coding on OPA1 protein. These lipoplexes are able to transport and efficiently deliver plasmid DNA into the cytoplasm thus inducing the recovering of normal mitochondrial phenotype in OPA1-Knockout MEFs. These lipoplexes show a good viability and high transfection efficiency as compared with other canonical transfer agents in *in vitro* experiments. Besides, these lipoplexes show a great bioaccumulation and transfection efficiency in different organs of CD-1 mouse model without any toxicity over time after intraperitoneal (IP), Intracardiac (IC) and intramuscular (IM) injection, as was confirmed by different techniques.

P4.4 Biophysical characterization of lipid-tagged peptides as fusion inhibitors for respiratory viruses

PATRICIA MORGADO DA-SILVA,^{1,§} Marcelo T. Augusto,¹ Matteo Porotto,² Anne Moscona,³ Nuno C Santos.¹

¹*Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal;* ²*Department of Pediatrics, Columbia University Medical Center, New York, USA;* ³*Departments of Pediatrics, Microbiology and Immunology, and Physiology and Cellular Biophysics, Columbia University Medical Center, New York, USA.*

§patriciamsilva@medicina.ulisboa.pt

Human parainfluenza viruses (HPIV) and respiratory syncytial virus (RSV) are among the most common respiratory pathogens affecting infants and children worldwide. Nowadays, acute respiratory infections are the leading cause of mortality in children under 5 years of age, accounting for nearly 20% of childhood deaths worldwide. There are no effective treatments available. Consequently, there is an urgent demand for efficient antiviral therapies. Infection of healthy cells requires fusion of the viral membrane with the target cell membrane, a process mediated by a trimeric viral fusion protein (F). Inhibitory peptides inhibit viral fusion by binding to F's intermediate, preventing it from advancing to the next step in membrane fusion. Here, we assessed variants of lipid-tagged F-derived peptides to search for properties that may associate with efficacy and broad-spectrum activity. Fluorescence spectroscopy was used to study the interaction of the peptides with biomembrane model systems. Using acrylamide, a quencher of tryptophan fluorescence, it was possible to understand the preferential localization of the peptides in lipid bilayers. The interaction of the peptides with human blood cell-binding was also evaluated using the dipole potential probe, di-8-ANEPPS. Understanding the membrane biophysics processes involved in enveloped viruses entry may enable the development of new therapeutic strategies.

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P5.1 Comparison of zika virus capsid protein with related flavivirus capsid proteins and their ability to interact with host lipid systems

ANA DE SOUTO-MARTINS,^{§,§} André Nascimento, André F. Faustino, Nuno C. Santos, Ivo C. Martins.

Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal.

[§]ana.martins@medicina.ulisboa.pt, [§]*EBSA bursary*

Zika (ZIKV), Dengue (DENV) and West Nile (WNV) viruses are related mosquito-borne flaviviruses. ZIKV infection was associated with severe congenital microcephaly in newborns and with Guillain-Barré syndrome. Although there is a lack of knowledge on basic aspects of the viral life cycle, much can be inferred from the closely related DENV and WNV. For example, DENV C interaction with host lipid droplets (LDs) is essential for viral replication, having been studied in detail by us. Thus, here, we investigated ZIKV C binding to host lipid systems via biophysical approaches. Zeta potential shows that ZIKV C interacts with intracellular LDs. However, ZIKV C-LDs interactions do not require potassium ions, as previously shown by us for DENV and WNV C. Dynamic light scattering measurements indicate that ZIKV C interacts with plasma lipoproteins, namely VLDL and LDL. ZIKV, WNV and DENV C proteins display similar predicted hydrophobicity, α -helical propensity and tertiary structure, which can thus be targeted via similar approaches. Combining this with our background on DENV C studies and pep14-23 development (an inhibitor of DENV C binding to host lipid systems, designed and patented by us), we will use this information in drug development strategies against ZIKV and related flaviviruses.

P5.2 A single-molecule manipulation assay to study the transcriptional dynamics of influenza a virus

CARLOS RODRIGUEZ-PULIDO,^{1,§,§} Rebeca Bocanegra-Rojo,¹ Rocío Coloma,² Jaime Martín-Benito,² Borja Ibarra.¹

¹IMDEA Nanociencia, Madrid, Spain; ²Centro Nacional de Biotecnología (CNB), Madrid, Spain.

§ carlos.rodriguez@imdea.org, § *SBE bursary*

Influenza A virus is an important human pathogen that causes yearly epidemics and occasional pandemics of flu. Because of its high mutation rate Influenza viruses evolve rapidly and are difficult to target with vaccines. On the molecular level, the dominant source of mutations is the error-prone RNA-dependent RNA polymerase, which is the protein complex responsible for replicating and transcribing the viral genome. We present an optical tweezers-based assay to establish the molecular and mechanistic processes that govern the real time kinetics of the viral transcription machinery at the single molecule level. This information is crucial to understand the effect of drugs on the operation of the viral transcription machinery at the molecular level and therefore, may have direct implication in the development of alternative therapeutic strategies against Influenza viruses.

P5.3 Liposome encapsulation of a fibrinolytic agent and its effect on clot degradation

PATRICIA M. CARVALHO,^{1,§} Mariana Teixeira,^{1,2} Diogo Sequeira,^{1,2} Nuno C. Santos,¹ Marco M. Domingues.¹

¹Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal; ²Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, Portugal.

§ pcarvalho@medicina.ulisboa.pt

There is increasing evidence for a consistent association of denser fibrin clot structure and higher resistance to degradation (fibrinolysis) with cardiovascular diseases (CVDs). CVDs account for nearly one-third of deaths worldwide and there is an urgent need to overcome this scenario. We aim to develop an encapsulated fibrinolytic lipid nanoparticle strategy with lower bleeding risk, to be incorporated in the clot structure. We studied the impact of the nanoparticle on clot formation and lysis and observed that the nanoparticles do not affect clot properties. Also, we concluded that the nanoparticle is stable over time without any measurable aggregation or change in surface charge. Turbidimetry studies showed that the presence of the nanoparticles reflected a non-significant small increase in fibrin fiber radius, protofibril packing and protein content with increasing lipid concentrations. Two methods of tPA encapsulation in lipid nanoparticles were tested, with one achieving 90% encapsulation efficiency. Ultracentrifugation was used to separate non-encapsulated material without triggering nanoparticle aggregation. Preliminary results have already demonstrated a controlled release of tPA in a solid emulsion of a clot, without activity loss. Future work will focus on optimizing the targeting element incorporation in the liposome surface.

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P6.1 Mechanism of permeation of ciprofloxacin copper-complex across the bacterial membrane

CARLA F. SOUSA,^{1,§,§} João T. S. Coimbra,² Maria J. Ramos,² Pedro. A. Fernandes,² Paula Gameiro.¹

¹REQUIMTE, LAQV, University of Porto, Porto, Portugal; ²REQUIMTE, UCIBIO, University of Porto, Porto, Portugal.

§cfilipams@gmail.com, §*SPBf bursary*

Ciprofloxacin (Cpx) is one of the most used Fluoroquinolones in clinical therapy. The increase in Cpx prescription in the last decades, and consequent misuse, brought an alarming increase in bacterial resistance found for this drug. Cpx acts inside the bacterial cell, by inhibition of topoisomerase enzymes, and its permeation in the cell is thus fundamental. Decrease in membrane permeability to this drug, by porin mutation, is an important mechanism of resistance. Cpx copper complexes (CuCpxPhen) have been studied as substitutes for Cpx. They should have an increased ability to cross the bacterial membrane, promoted by favourable interactions between the positively charged copper complexes and the negatively charged lipid polar heads. In this work, we studied the permeation of Cpx and CuCpxPhen in a phosphatidylglycerol model membrane that mimics the negatively charged bacterial membrane. Fluorescence Spectroscopy and Molecular Dynamics were used to determine the partition coefficient of the drugs and to further explore the interactions that are involved in the permeation process. The investigation of CuCpxPhen permeation mechanism and comparison with Cpx provide insights that will be significant to proceed with the study of metal-complexes of fluoroquinolones as alternatives in resistant infections.

P6.2 DNA crookedness regulates DNA mechanical properties at short length scales

ALBERTO MARÍN-GONZÁLEZ,^{1,§,§} Vilhena J. Guilherme,² Fernando Moreno-Herrero,¹ Rubén Pérez.³

¹Department of Macromolecular Structures, Centro Nacional de Biotecnología (CNB-CSIC), Madrid, Spain;

²Department of Physics, University of Basel, Basel, Switzerland; ³Departamento de Física Teórica de la Materia Condensada, Universidad Autónoma de Madrid, Madrid, Spain.

§amarin@cnb.csic.es, §Best poster prize, SBE bursary

Sequence-dependent DNA conformation and flexibility play a fundamental role in specificity of DNA-protein interactions. Here we quantify the DNA crookedness: a sequence-dependent deformation of DNA that consists on periodic bends of the base pair centers chain. Using 100 microsecond-long atomistic molecular dynamics simulations, we found that DNA crookedness and its associated flexibility are bijective: unveiling a one-to-one relation between DNA structure and dynamics. This allowed us to build a predictive model to compute DNA stretching stiffness from solely its structure. Sequences with very little crookedness show extremely high stiffness and have been previously shown to form unstable nucleosomes and promote gene expression. Interestingly, the crookedness can be tailored by epigenetic modifications, known to affect gene expression. Our results rationalize the idea that the DNA sequence is not only a chemical code, but also a physical one that allows to finely regulate its mechanical properties and, possibly, its 3D arrangement inside the cell.

P6.3 Numerical procedure to calculate the profile of oxygen tension and flow into the cornea as a function of the oxygen tension at the interface cornea-lens

ANDREU ANDRIO-BALADO,^{1,§} Marcel Aguilera-Arzo,¹ Vicente Compañ-Moreno.²

¹Universitat Jaume I, Castellón, Spain; ²Universitat Politècnica de Valencia, Valencia, Spain.

§andrio@uji.es

The mathematical models used to determine the oxygen consumption in the cornea have been based on determining the oxygen partial pressure in the cornea-tear interface. However, they have been frequently misunderstood. All these models describe the oxygen consumption in the cornea as a function of pressure $Q_c(p_c)$. Traditionally oxygen consumption was considered constant through the cornea since the diffusion equation was treated in a stationary state. This approximation lead to negative values of oxygen tension in the cornea, which is unphysical [1–4]. Others have developed models in which the oxygen consumption $Q_c(p_c)$ is described by means of the Sigmoidal Model (Alvord *et al.*, 2007) [5]. Finally, others have opted for the Metabolic Model, describing $Q_c(p_c)$ as a consequence of aerobic metabolism [6–8]. In this work, we report the oxygen concentration profiles and flows through the system formed by cornea-tears-lens using different models of oxygen consumption in the cornea region, by numerically solving the diffusion equation. The oxygen concentration profiles and flows depend on the oxygen tension at the interface cornea-lens, and also on the physical characteristics of the different tissues and lens. We discuss all these models within our numeric framework.

P6.4 Assessment of the internal dose in a voxelised human phantom using Geant4

BHAR MAROUA,^{1,§} Manai Kais,² Omrane Kadri.³

¹*Institut Supérieur des technologies médicales, Université Tunis El Manar, Tunisia;* ²*Unité de Physique Nucléaire et des Hautes Energies, Faculté des Sciences de Tunis, Université Tunis El Manar, Tunisia;* ³*Department of Radiological Sciences, College of Applied Medical Sciences, King Saud University, Kingdom of Saudi Arabia.*

§bhar.maroua@yahoo.com

The computational human phantom study had a great of interest in various fields such as: medical imaging, radiation protection, radiotherapy. . . . All has been developed in the main objective to assess the absorbed dose in deferent organs. This quantity can't be directly measured due to the complexity of human anatomy and the inhomogeneity in body structures. So his determination can then serve to compute organ equivalent dose and whole-body effective dose. The voxel phantom 'High-Definition Reference Korean-Man (HDRK-Man)' was implemented into the Monte Carlo transport toolkit Geant4. The assessment of the averaged absorbed dose for paired organs separately using Monte Carlo calculation is still limited. To overcome this limit, this work focuses on the calculation separately of the pulmonary averaged absorbed dose in the right and left lung during X-ray examination procedures. The percentage difference between the right and left lung doses (ΔD) was computed in different angles of incidence. Results were compared to those obtained previously using other simulation code, and good agreement was observed which allows to deepen the study. Given the accuracy of our results, it can be considered as a complement of the medical physicist task in the quality control tests and radiation protection dosimetry.

P6.5 Vibrational spectra in harmonic and anharmonic approximation of the two isomers of resveratrol

REKA-ANITA DOMOKOS,^{1,§} Vasile Chis.²

¹*Department of Biomolecular Physics, Faculty of Physics, Babes-Bolyai University, Cluj Napoca, Romania;* ²*Babes-Bolyai University, Cluj Napoca, Romania.*

§rekadomokos27@gmail.com

Resveratrol (3,4',5 – trihydroxystilbene, RESV) has been proven to be a strong antioxidant and anti-inflammatory compound used to treat diseases like heart failure, breast and prostate cancers, stroke, brain damage, etc. Moreover, recent studies demonstrated that RESV is a promising chemo-preventive agent able to extend lifespan in several organisms. In this work we present a joint experimental and theoretical study on the vibrational spectra features of *trans*- and *cis* isomers of RESV. FT-Raman spectra of the two isomers were recorded and assigned based on DFT calculations, using both the harmonic and anharmonic approximations. Detailed features of the Raman spectra have been explained and characteristic vibrational bands for the two isomers have been clearly identified. The excellent correlation between the theoretical results and experiment guarantees the reliable vibrational characterization of the investigated compounds. Moreover, it is demonstrated that a clear discrimination of the two isomers is possible based on Raman technique coupled to quantum chemical methods.

P6.6 Molecular dynamics simulations at the interface between water and proteins: Power and infrared spectra

JOSÉ ANGEL MARTÍNEZ-GONZALEZ,^{1,2,§} Prithwish Nandi,³ Zdenek Futera,² Niall English,² Aoife Gowen.¹

¹*School of Biosystems and Food Engineering, University College Dublin, Belfield, Dublin, Ireland;* ²*School of Chemical and Bioprocess Engineering, University College Dublin, Belfield, Ireland;* ³*Irish Centre for High-End Computing, Trinity Tecnology and Enterprise Campus, Dublin, Ireland.*

§jose.martinez-gonzalez@ucd.ie

The water molecules near the interface have a confined environment that manifests itself in variations of their vibrational properties, and in the resultant infrared spectrum. In this communication, we explain our theoretical approach based on molecular dynamics simulation to characterise the water-protein interaction and obtain the power and infrared spectrum. We performed Molecular Dynamics simulations using GROMACS package with OPLS and Ferguson flexible water model for proteins (ubiquitin and hen egg-white lysozyme) and water, respectively. All simulations were carried out at constant temperature and pressure, and the smooth particle-mesh Ewald (SPME) method for the long-range electrostatic interaction. Any constraint algorithm for hydrogen vibration was used. Fourier transformation was applied to calculate the velocity auto-correlation function, which allows the power spectra to be obtained. IR spectra for all systems were obtained via the electrical flux approach. Power spectra and IR spectra for the proteins water interface have been compared to ice and liquid water structure using the same water model in order to find similarities.

P6.7 From molecular dynamics simulations to genetic interpretation: How about trying to describe the complete proteome mutational space?

JUAN JOSÉ GALANO-FRUTOS,^{1,§} Héctor García-Cebollada,² Javier Sancho.¹

¹*Biochemistry and Molecular and Cellular Biology Department, Faculty of Sciences, University of Zaragoza, Zaragoza, Spain;* ²*Biocomputation and Complex Systems Physics Institute (BIFI). Joint Unit BIFI-IQFR (CSIC), Zaragoza, Spain.*

§juanjogf@gmail.com

Nowadays genetic variants are easy to identify. Being able to provide conclusive diagnoses on their pathogenicity is crucial. Advances in the description of genomes, protein stability understanding, and protein dynamics simulation are illuminating the relationship between mutation and disease. Likewise, big efforts are being done to massively solve protein structures at atomic resolution. All this cumulated knowledge represents an opportunity to address the prediction of mutation effects on protein functionality from an atomistic molecular dynamics (MD) point of view. Here, we aim at developing a methodology based on the analysis of relaxation (unfolding) MD trajectories to accurately assess the compatibility of a given mutation with the function associated to the bearing protein. The feasibility of such a methodology is being explored using well-established mutational/pathogenic relationship cases. Such an approach may provide valuable insights to complement and improve the predictions offered by current applications that heavily rely on sequence-conservation analyses, e.g. SIFT and PolyPhen-2. With this perspective, we will show a correlational analysis of the simulation relaxation times for two structural types of proteins (alpha and beta) in order to project the time required to address the complete proteome mutational space with current computational resources.

P6.8 Introducing critical pain related genes: A system biology approach

MAHSHID SABERI,^{1,§} Mohammad Mahdi Zadeh-Esmaeel,² Mostafa Rezaei-Tavirani.³

¹Science and Research Branch, Islamic Azad University, Tehran, Iran; ²Department of Computer and Engineering and Information Technology, Amirkabir University of Technology, Tehran, Iran; ³Proteomics Research Center, Faculty of Paramedical Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

§Mahshid.saberi@yahoo.com

Background. Pain has valuable importance in diagnosis and also distress of patients. many molecular reagents are introduced which are related to pain. in this research the pain related genes screen to identify the critical genes. **Methods.** Pain related genes were pulling out from string database and cytoscape software was used to make interactome unit. the central genes and their neighbors were analyzed. the genes were clustered and the crucial genes were introduced. **Results.** 159 genes of network were analyzed and fos, il6, tnf, tac1, il8, and kng1 were identified as crucial genes. more analysis revealed that 88 genes are connected directly to the central genes. more resolution led to ignoring tnf and il8 and considering scn9a and paics as additional critical nodes. **Conclusion.** Eight critical pain related genes and possible therapeutic targets were identified.

P6.9 Effect of interfacial charge on the conductance of protein nanopores. A structure-based computational approach

MARCEL AGUILELLA-ARZO,[§] Antonio Alcaraz, María Lidón López-Peris, Vicente M. Aguilera.

Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Castellón, Spain.

§arzo@uji.es

We investigate here the effect of surface charge on the conductive properties of protein nanochannels. The equations that describe ion electrodiffusion (Poisson-Nernst-Planck equations) are solved numerically [1] in two protein channels inserted in model membranes, using as input their atomic three-dimensional structure (obtained from the *Protein Data Bank*). We explore the bacterial porin OmpF, which is a trimer of intermediate pore size and gramicidin A, a traditional example of narrow channel, both widely used in studies of electrodiffusion as model systems. We find that membrane charges have a different impact on each protein. For the wide channel OmpF there is a reduction of the so-called access resistance, but also of the resistance of the channel itself. However in the case of a narrow channels like gramicidin A only the channel resistance is reduced, without any important effect on the interfacial solution. Other consequences are also discussed in light of our results.

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P6.10 Surface charge and fatty acids modulate the membrane permeation rate of cell-penetrating-peptides: Adsorption energy counterbalances pore formation cost

MATÍAS ALEJANDRO-VÍA,^{1,§} Natalia Wilke,² Mario Gabriel del Pópulo.³

¹*Instituto de Histología y Embriología de Mendoza, CONICET, Argentina;* ²*Centro de Investigaciones en Química Biológica de Córdoba, UNC-CONICET, Córdoba, Argentina;* ³*Facultad de Ciencias Exactas y Naturales, Universidad Nacional de Cuyo, Argentina.*

§matysvia@hotmail.com

Cell-penetrating-peptides (CPPs) are rich in basic aminoacids, which make peptides charged and hydrophilic at physiological pH. Following these traits, the observations that CPPs translocate lipid bilayers conferring cell-internalization ability to membrane impermeable cargos are counterintuitive and deserve scrutiny at the molecular level. Membrane properties, such as lipid composition and surface charge, regulate the rate and efficiency of the translocation process. In this work, we use Molecular Dynamics simulations and free-energy calculations to demonstrate that the presence of fatty acids and other negatively charged lipids in the membrane boosts the translocation rate of a typical CPP. We focus on Arg9, and compare its translocation efficiency with that of a hydrophilic but non-CPP peptide, Gly9. We also show that when the latter is chemically bound to Arg9, Arg9-Gly9 crosses the membrane with a lower free energy cost than Gly9 alone. We explain our findings through a simple phenomenological model that considers the energy gained upon adsorption of the translocating molecule, and the energy barrier that must be surmounted to bring the molecule from the surface to the centre of the bilayer. Our simulations show that sufficiently strong binding to the membrane surface and negatively charged fatty acids enhance the so called CPP-effect.

P6.11 Biophysical characterization of E336k mutant of the human apoptosis inducing factor

PATRICIA FERREIRA-NEILA,[§] Mikel Marín-Baquero, Silvia Romero-Tamayo, Adrian Velázquez-Campoy, Milagros Medina.

Department of Biochemistry and molecular and cell Biology and Institute of Biocomputation and Physics of Complex Systems, University of Zaragoza, Zaragoza, Spain.

§ferreira@unizar.es

The human Apoptosis Inducing Factor (hAIF), a caspase-independent cell death inductor, plays a vital function in mitochondria contributing to the biogenesis of respiratory complexes (1, 2). In mitochondria, hAIF is present in a monomer-dimer equilibrium modulated by the NADH/NAD⁺ levels. The reduced hAIF-coenzyme complex binds two independent NADH molecules per protein protomer: in the NADH-binding domain and in the apoptotic region (3). Different hAIF allelic variants have been identified causing neurodegenerative disorders associated with several mitochondrial adenopathies (4). Residues G308 and G338 (involved in pathogenic mutations), together with E336 stabilize the adenine moiety of the catalytic NADH molecule. We produced mutant E336K to evaluate the relevance of this residue in the hAIF activities. Here, we have particularly addressed its role in hAIF redox properties by performing protein stability, kinetic and ligand binding studies. These studies contribute to better understand new rare diseases in which AIF plays a key role in neuronal death via its moonlighting activity.

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P6.12 How nanoscale protein interactions determine the mesoscale dynamic organisation of bacterial outer membrane proteins

ANNA L. DUNCAN,^{1,§} Matthieu Chavent,^{1,2} Patrice Rassam,^{1,3} Oliver Birkholz,⁴ Jean Hélie,¹ Tyler Reddy,¹ Dmitry Beliaev,⁴ Ben Hambly,⁵ Jacob Piehler,⁴ Colin Kleanthous,¹ Mark S. P. Sansom.¹

¹Department of Biochemistry, University of Oxford, UK; ²IPBS, Université de Toulouse, CNRS, UPS, Toulouse, France; ³Université de Strasbourg, France ; ⁴Department of Biology, University of Osnabrück, Germany; ⁵Mathematical Institute, University of Oxford, UK.

§anna.duncan@bioch.ox.ac.uk

The spatiotemporal organization of membrane proteins is often characterised by the formation of large protein clusters. In *Escherichia coli*, outer membrane protein (OMP) clustering leads to OMP islands, the formation of which underpins OMP turnover (1) and drives organization across the cell envelope (2). Modelling how OMP islands form in order to understand their origin and outer membrane behaviour has been confounded by the inherent difficulties of simulating large numbers of OMPs over meaningful timescales. Here, we overcome these problems by training a mesoscale model incorporating 1000s of OMPs on coarse grained molecular dynamics simulations. We achieve simulations over timescales that allow direct comparison to experimental data of OMP behaviour. We show that specific interaction surfaces between OMPs are key to the formation of OMP clusters, that OMP clusters present a mesh of moving barriers that confine newly inserted proteins within islands, and that mesoscale simulations recapitulate the restricted diffusion characteristics of OMPs in vitro.

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“DNA crookedness regulates DNA mechanical properties at short length scales,” **P6.2.**

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Univ. Lisboa, PT.

“Exploring the membrane activity of a designed antimicrobial peptide with fusogenic properties,” **S2.1.**



NICOLÁS SEBASTIÁN GONZÁLEZ-FOUTEL,
IIB-CONICET, San Martín, AR.

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Tribute | Carlos Gómez-Moreno

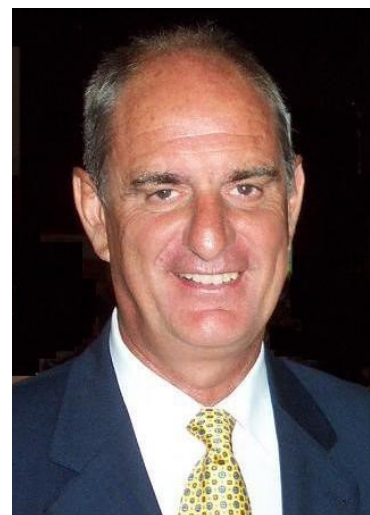
MILAGROS MEDINA, JAVIER SANCHO.

Departamento de Bioquímica y Biología Molecular y Celular y BIFI. Universidad de Zaragoza, Spain.

When CARLOS GÓMEZ-MORENO assumed his *Cátedra* (full Professorship) at the University of Zaragoza (1983) he may not have imagined the future of the Structural Biology and Biophysics in this city. This makes us look back to see the very long road that he has travelled and that, fortunately for us, we have in many occasions intensely walked with him. CARLOS has lived, and keeps living, by pushing on each of the turns of life to come out in the best possible way; forcing himself to work hard, to make his ideas and actions valuable for all of us.

He did his doctorate in Seville, moving later to the USA for two postdoctoral periods, first in Ohio and later in San Francisco. As he likes to say:

“ Four years, two postdocs, two sons, many experiences, much of training, a new vision of life and ideas in science to develop onwards, first in a short period in Granada, and later, for 35 years in Zaragoza, where I developed most of my scientific life, as well as my family and personal life. ”



Prof. Carlos Gómez-Moreno

In 1983 CARLOS arrived to the recently founded Department of Biochemistry and Molecular and Cell Biology at the University of Zaragoza (DBMCB-UNIZAR), with 35 years, a brand new full Professorship and a test tube containing a sample of *Anabaena variabilis*, the microscopic organism on which he has been working during all this time. When he came to Zaragoza, he joined a department that was active in two lines of research, metabolism of lipids and cell biology, both of them very different from his own expertise. In this context, he founded his research group with the biological material he brought and with many ideas to elucidate the reaction mechanisms of photosynthetic enzymes. This laid the foundations of Structural Biology and Biophysics at the University of Zaragoza and in the whole region of Aragón, and some of us had the fortune to be there almost from the very beginning. One of us (JAVIER) joined first, and as CARLOS recalls, “he was not only a brilliant student, but also strong and able to handle the 600 liters of cultures that we needed to produce proteins, working long days, if you were feeding him well.” He also attracted to this nascent group a new lecturer in Plant Physiology at DBMCB, MARIA LUISA PELEATO, and continued with his “psychological” task to convince the rest of us to carry out our PhD under his supervision. In his first 10 years in Zaragoza, MARIA FILLAT, JOSÉ JAVIER PUEYO, MILAGROS MEDINA and TERESA BES, in addition to JAVIER SANCHO, got their PhD in CARLOS’ group, and many more scientists came later on. These were the fruitful seeds that he planted for



CARLOS GÓMEZ-MORENO addresses the public at the "Special Recognition" act during the 6th IIBC / X ICB 2018 in Castellón. Left to right, CARLOS GÓMEZ-MORENO, VICENTE AGUILLELLA, JESÚS PÉREZ-GIL, NUNO SANTOS and ANTÓNIO J. DA COSTA-FILHO

Structural Biology and Biophysics in Zaragoza, since most of us have made our scientific career and created our own research groups in these areas, in many cases remaining at UNIZAR, thus spreading the field in the form of numerous talented CARLOS' "grandsons" and "granddaughters" PhDs, doctorated with us.

He can also be very proud of having contributed to the development of two biotechnological companies, based in Aragón, that today are selling to hundreds of countries and employing many people trained at UNIZAR. In CARLOS own words:

“ I have fulfilled the main objectives of a University Professor: To educate people, who help improving the living conditions of their territory, through the development of knowledge and entrepreneurship. ”

But he did not only plant the seeds, he has also remained watering them along these 35 years. From the very beginning he showed us the importance to open up to the world. He started collaborations with different groups in the USA and Europe, which helped us, young people at that time, to visit other laboratories, learn new techniques and meet illustrious scientists; who, in turn, made us realize that our work was relevant. CARLOS also showed us that "not everything is abroad." Indeed, he has been strongly committed with the Spanish science in general, and particularly at UNIZAR and with national Bioscience related Societies. Among them, our Spanish Biophysical Society (SBE) has been very important for him. In fact, as we learnt from FELIX GOÑI at the 2018 IIBC Congress in Castellón, CARLOS was there from the very beginning and he has always transmitted to us the importance of supporting the societies. Thus, since the beginning of our careers he had the grace to introduce us to his/her respected colleagues, in such a sympathetic way that made us feel their respect, support and friendship until today. At the local level, he also contributed to the foundation and development of new research institutes at UNIZAR, particularly, the Institute for Biocomputation and Physics of Complex Systems (BIFI) and more significantly the Institute of Nanoscience of Aragon (INA).

Some of us also have had the privilege to continue working closely with CARLOS during the last 25 years, particularly MARTA MARTÍNEZ-JÚLVEZ and myself (MILAGROS). During this time we implemented fast kinetic methods and other biophysical tools to characterize the mechanisms of interaction between electron-transfer proteins from the photosynthetic chain and also actively

worked to obtain X-ray crystal structures from our biological molecules. Always opening new frontiers, more recently, and together with ANA ISABEL GRACIA-LOSTAO, CARLOS research activity has been primarily aimed at the use of atomic force microscopy for the study of interactions between proteins at the single molecule level.

At the point of slowing down his scientific career, CARLOS must be proud of himself, not only because of the many things he achieved in Science, but also because at the same time he has been hard-working, noble, loyal and generous to us. We know for sure that he will keep looking at the future with the same passion and personality that he has shown along his professional life.

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On the 6th International Iberian Biophysics Congress & X Iberoamerican Congress of Biophysics

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On the 6th International Iberian Biophysics Congress & X Iberoamerican Congress of Biophysics

VICENTE M. AGUILLELLA.

Laboratory of Molecular Biophysics, Department of Physics, Universitat Jaume I, Castellón, Spain.

Chair of the Organizing Committee.

The XVII Annual Meeting of the Spanish Biophysical Society (SBE) took place at the Campus of Universitat Jaume I in Castellón, on June 20–22, 2018. This year, the SBE, the Portuguese Biophysical Society (SPBf) and the Latin American Federation of Biophysical Societies (LAFeBS) jointly organized this Scientific Conference, so that it was named as 6th International Iberian Biophysics Congress and X Iberoamerican Congress of Biophysics.

Over 200 scientists, from nearly 20 countries, gathered in Castellón to discuss the state-of-the-art in Biophysics during three days. They presented nearly 170 communications, 70 of them in Talks and around a hundred Posters in two sessions. As expected, young participants (PhD students and postdocs) were by far the major part of the audience. Their participation was greatly facilitated by nearly 40 bursaries kindly offered by the SBE, the SPBf, the European Biophysical Societies Association (EBSA), the International Society of Magnetic Resonance (ISMAR) and the Biophysical Society International Relations Committee.



Group picture of 2018 IIBC attendees.



Pianist PATRIZIA MARCATELLO and clarinetist ANTONIO AMANTI.

Molecular Biophysics. Renowned speakers delivered five Plenary Lectures. SERGEY BEZRUKOV, JORDI GARCÍA OJALVO, ROSANGELA ITRI, CELERINO ABAD ZAPATERO and FRED MACKINTOSH stimulated the discussion during and after their talks. The first day was devoted to a special session on *New and Notable Biophysics* —with speakers selected by their recent outstanding contributions— followed by a new stand alone symposium on *Physics in Biology*. With this symposium, highly successful looking at the number of abstracts submitted, and with the selection of the Plenary Speakers, we wanted to highlight this year the key role of Physics for quantitative understanding of biological problems, not only focused in the Experimental Methods and Simulation but also, and particularly, on the theory. As some highly reputed biophysicists wrote,

“ The connection between biology and physics is a two way street. However, the heavy traffic has gone one way. Many tools from physics have been adopted by researchers in the biological sciences. The return traffic, where biological ideas motivate physical considerations, has been less visible. ”

Three of the plenary lectures focused on this *return traffic* from different perspectives: diffusional transport of single particles, electrical signaling in bacteria and phase transitions were the topics chosen by the speakers. The RSEF-SBE plenary lecture took a step further in strengthening the ties between the two Spanish societies and attracting towards the SBE physicists who are actually working on biological problems.

Not all activities were strictly scientific. At the end of the first Symposium, on the very first day of the Meeting, the participants could enjoy a Clarinet and Piano recital, sponsored by the *Small Biosystems Lab* from the University of Barcelona, performed by two talented Italian musicians, the clarinetist ANTONIO AMANTI and the pianist PATRIZIA MARCATELLO. Prof. FÉLIX RITORT introduced the artists and the repertoire: Clarinet Concerto No.1 in F minor by CARL MARIA VON WEBER; Sonata in B-Flat Clarinet and Piano by FRANCIS POULENC and Sholem-alekhem, Rov Feidman for Clarinet and Piano by BELA KOVACS. After this relaxing session, there was ample time for networking during the welcoming reception, although some participants rushed to the nearby classrooms to watch on TV the a football match of the Spanish national team participating in the World Cup.

This Congress edition awarded a prize to the three best posters, sponsored by FEBS Letters, Biopolymers and Peptide Science, respectively, and the Imagin' Action prize sponsored by Hamamatsu. There was also an award to the best oral communication presented by a young researcher member of the SBE or SPBf, offered by the *Luis de Camoens Chair* of the University Carlos III of Madrid, together with Banco de Santander and Ramón Areces Foundation. Three SBE awards (Bruker-Manuel Rico, Enrique Pérez-Payá and SBE-33) and the SPBf award to the best Young

Researcher recognized the scientific trajectory of four outstanding researchers in our field, who delivered their talks in the Awards Symposium on Friday 22. During the closing ceremony, the SBE payed a warm tribute to one of his members, Prof. CARLOS GÓMEZ-MORENO, from the University of Zaragoza, for his long scientific trajectory and contributions to the advance of Biophysics in Spain.

As Chair of IIBC-2018, I would like to thank all participants in the Meeting. In particular to the chairs of the sessions, who selected an excellent list of speakers for their respective symposia. All of them contributed to setting high standards in the scientific level of the Meeting. Also the collaborative effort of all members of the Laboratory of Molecular Biophysics in Universitat Jaume I was essential the preparation and development of the Congress. In addition, I would like to thank ALÓ Congress SL, for their support with logistics, Technical Secretary and Website of the Congress. Last, but not least, I could not forget to mention SBE and SPBf for their support and Universitat Jaume I for the use of its facilities. There were several Institutional and Commercial sponsors that made possible the celebration of the Meeting. I am deeply grateful for their economic support: Generalitat Valenciana, Ministerio de Ciencia, Innovación y Universidades, UJI, Small Biosystems Lab, EBSA, ISMAR, Biophysical Society, Cátedra Luis de Camoens UCIII (B. Santander and Areces Foundation), Nanion, Wyatt Technology, Dynamic Biosensors, Elsevier, iesmat, LabClinics, NanoTemper, Hamamatsu, Lasing, Paralab BIO, AntalGenics, PrimaDerm, BCNPeptides and Bruker.



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